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MOLECULAR MECHANISMS OF EFFECTS OF BOTULINUS AND TETANUS NEUROTOXINS

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[Text] Physicochemical properties of toxin molecules, significance of different amino acids to toxicity and role of gangliosides in chemical reception of toxins are discussed. Analysis is made of distinctions of presynaptic effects on the example of central and peripheral synapses. Evaluation is made of effects of toxins on main processes involved in synaptic transmission.

Introduction

The mechanisms of effects of two extremely potent neurotoxins--botulinus and tetanus--are of considerable interest to researchers. The biologist and toxicologist is concerned with the striking toxicity of these proteins, as compared to nonprotein toxic agents. The interest of medical figures in the mechanism of effects of these toxins is attributable to the need to work out pathogenetic therapy of botulism and tetanus, which are diseases associated with a rather high mortality rate. Neurophysiologists use these toxins to investigate mechanisms of synaptic transmission, since they are quite selective for certain types of chemical synapses. For researchers concerned with the denervation syndrome, botulism and localized tetanus are convenient models of chemical denervation.

We shall discuss here the structural distinctions of molecules of these toxins, the specifics of their effects and biochemical changes in stricken tissues.

Physicochemical Properties of Tetanus and Botulinus Toxins

Tetanus toxin (TT) is a simple protein. Sedimentation and chromatographic studies indicate that the molecular mass of TT is close to 150,000 dalton (Raynaud et al., 1960; Dawson, Mauritzen, 1967; Murphy, Miller, 1967; Mangalo et al., 1968; Murphy et al., 1968; Dawson, Nickol, 1969; Bizzini et al., 1973a; Robinson et al., 1975; Matsuda, Yoneda, 1976). Preliminary data have been obtained on the dimensions and shape of TT molecules: Stokes' radius 4.74 (Bizzini et al., 1973a), friction ratio 1.09 (Mangalo et al., 1968) and 1.5 (Dawson, Nickol, 1969).

Studies of amino acid composition failed to demonstrate unusual amino acids (Murphy et al., 1968; Dawson, Mauritzen, 1967). The high levels of aspartic and glutamic acid determine acidity of protein (Pillemer, 1948). The only terminal amino acid is leucine (Bizzini et al., 1970) or glycine (Holmes, Ryan, 1971). Estimation of TT polarity on the basis of polar amino acids in the molecule shows that the index of TT polarity is of the same order as in other soluble proteins--hemoglobin, insulin, lactate dehydrogenase--and it is higher than in membrane proteins (Robinson et al., 1975).

SH groups are demonstrable in the TT molecule only after denaturation: four after treatment with urea and two more after combined treatment with urea and 8-hydroxyquinoline sulfate (Murphy et al., 1968; Bizzini et al., 1970). Four of the SH groups form two S-S bridges in the molecule of unadulterated TT, one in each subunit (Craven, Dawson, 1973).

Studies of products of denaturation of intracellular TT using molecular sieves, electrophoresis in polyacrylamide gel and ultracentrifugation warranted the assumption that TT is a dimer with molecular mass of ~140,000 dalton, the monomer units of which are joined by noncovalent bonds. In turn, each monomer contains one light and one heavy chain (21,000 and 52,000 dalton, respectively) connected by a single S-S bond (Bizzini et al., 1973a). On the other hand, convincing evidence has been offered of the fact that TT is a solitary polypeptide chain (Murphy, Miller, 1967), the structures of extracellular (filtrate) and intracellular TT being different. After dissociation of disulfide bonds, only the extracellular toxin separates into two fragments (95,000 and 55,000 dalton--Craven, Dawson, 1973). The amino acid composition, number of SH and S-S groups, toxicity and immunogenicity of extracellular and intracellular TT are the same (Bizzini et al., 1970; Craven, Dawson, 1973). It was subsequently shown that dissociation of sulfide-treated molecules of extracellular and intracellular TT yields similar products of breakdown of the heavy and light chain (Dawson, 1975).

It was established (Matsuda, Yoneda, 1974) that intracellular TT is a single polypeptide chain with molecular mass of 160,000 dalton. Unlike extracellular TT, intracellular TT does not break down into components of 107,000 and 53,000 dalton under the influence of dithiotreitol and sodium dodecylsulfate. After trypsin treatment, such dissociation was also found in intracellular TT. In accordance with the assumption that intracellular TT is an inactivated form of extracellular TT, the authors discovered 3-fold increase in toxicity of intracellular TT after trypsin treatment. Later on, the same authors succeeded in restoring the original TT molecule, using an equimolar mixture of light and heavy fragments (Matsuda, Yoneda, 1976).

Analysis of the spectrum of circular dichroism of TT solution revealed that there are maximums at 208 and 217 nm and an arm at 223 nm. A comparison of data concerning ellipticity in the 200-250 nm region to the corresponding values for peptides with known conformation warranted the conclusion that 20% of the TT molecule is represented by an α spiral and 23% by a β structure. Tryptophan, tyrosine and phenylalanine make an appreciable contribution to the spectrum in the region above 250 nm. There is more rotation near the main bands of circular dichroism in TT than peptides containing residues of aromatic amino acids, which is indicative of an orderly secondary structure (Robinson et al., 1974).

Botulin toxin (BT): There are several serologically different types of BT (A, B, C_1, C_2, D, E, F and G) that strike primarily specific animal species. All BT are simple proteins. When isolating BT from microorganisms of different strains using methods that preclude aggregation and disaggregation products are obtained that have sedimentation constants of 12S and 16S (for A and B lines) and 10-12S (for E and F lines). When acidulated, these products split into two components, and in all instances the toxic components have a molecular mass of ~150,000 dalton (Sakaguchi et al., 1974). Analogous figures were obtained for the toxic component in another laboratory (Boroff, DasGupta, 1971). The behavior of BT under electrophoresis (Kitamura et al., 1967) and chromatography on ion exchange resins (Boroff, DasGupta, 1971) indicates that all types of BT are acid proteins. The structure of BT is very similar to that of TT. Extracellular BT consists of a heavy and light polypeptide chain (molecular masses of 100,000 and 50,000 dalton, respectively) connected by an S-S bridge. Another S-S bridge was demonstrated in the heavy chain (DasGupta, 1979).

Toxophore Grouping of TT and BT

Investigation of formol detoxification yielded some information about the nature of toxophore groups and role of structure of the TT molecule. In several studies (Blass et al., 1965, 1967, 1969), tetanus anatoxin was submitted to hydrolysis, and amino acid composition was analyzed. As compared to TT, acid hydrolysates of formol anatoxin showed a decrease in tyrosine and lysine, and compounds were demonstrated, some of which could be identified. One of them has two lysine residues connected by methylene bridges; in others lysine is joined by methylene bridges to the phenol ring of tyrosine in position 3 or positions 3 and 5. Hence, it was concluded that tyrosine and lysine residues are contained in the toxophore center. Another explanation was the assumption that toxophore areas were obscured after formation of methylene bridges (Bizzini et al., 1974). It was demonstrated that formol detoxification leads to conformation changes near the tyrosine, tryptophan and phenylalanine residues.

A study dealing with the significance of lysine residues to tetanus toxin toxicity demonstrated that partial maleylization is associated with loss of toxicity and antigenicity, which is apparently due to a change in charge and conformation changes in the TT molecule. Partial demaleylization is associated with some restoration of immunogenicity. Amidation and reducing alkylation, which do not alter appreciably the charge of the TT molecule, caused decline of toxicity only when there was modification of a significant number of lysine residues (Bizzini et al., 1975). When using other methods of modification of lysin, it was possible to obtain distinct detoxification, even without altering conformation of TT (Robinson et al., 1975). Regardless of which method was used to modify lysine residues--reduction alkylation (Stein, Biel, 1973), methylation (Bizzini et al., 1975), amidation (Bizzini et al., 1975), carbamylation (Robinson et al., 1975), detoxification was demonstrable only after modification of 40-85% of the lysine residues in the TT molecule, which speaks against the hypothesis of lysine residues present in the toxophore center.

Photooxidation of tryptophan radicals with exposure to ultraviolet light in the presence of methylene blue (Stein, Biel, 1973) or modification of 10 out of

13 tryptophan residues with Koshland reagent (2-oxy-5-nitrobenzyl bromide--Bizzini et al., 1973) detoxifies TT. Consequently, tryptophan residues are important to maintaining toxicity. This also applies to histidine residues. Treatment of TT with ethoxy formic anhydride at pH levels that cause mainly modification of histidine residues inactivates TT (Bizzini et al., 1973).

Nitration of only 6 out of 81 tyrosine residues in the specific reaction with tetranitromethane elicits complete detoxification of TT (Bizzini et al., 1973). This fact is apparently direct evidence of involvement of tyrosine residues in the toxophore center, if not concurrent loss of capacity of nitrated toxin to bind with nerve tissue. The above data do not enable us to draw an unequivocal conclusion about the participation of a given amino acid in formation of the toxophore center. The data pertaining to tyrosine residues appear to be the most convincing.

The very first studies of toxophore groups of BT revealed that chemical modification of amino groups leads to loss of toxicity (Shantz, Spero, 1957). Ketene treatment led to rapid loss of toxicity, and this was a first order reaction. Since the ketene reacts with free amino acids, the hydroxyl group of phenol and free SH groups, there was indirect assessment of the role of the modified groups. Loss of free amino acids was observed throughout the detoxification process, whereas o-acetylation was manifested only after significant loss of activity. Rejecting involvement of SH groups (iodobenzoic acid did not elicit detoxification), the authors arrived at the conclusion that toxicity is related to α-amino acids or a few ε-amino groups. In any case, there had to be a small number of amino groups related to toxicity.

Studies of photooxidation of BT in the presence of methylene blue revealed virtually 100% loss of toxicity within 5 min (Weil et al., 1957). Since photooxidation modifies histidine, tryptophan, cysteine, tyrosine and methionine residues, it is impossible to relate detoxification of BT to modification of specific amino acid residues. Nevertheless, the authors assumed that toxicity is attributable to histidine residues.

The hypothesis had been advanced (Boroff, 1959) that BT toxicity is determined by tryptophan residues. It is known that proteins containing aromatic amino acids (tryptophan, tyrosine, phenylalanine) show fluorescence when exposed to light in the UV [ultraviolet] range (285 nm). All modifications of BT structure leading to depression of fluorescence at 350 nm (maximum of the spectrum of tryptophan fluorescence) elicited inactivation of BT.

Photooxidation at pH 3.9, which modified only tryptophan, methionine and cysteine residues, also inactivated BT. A significant decrease in methionine and tryptophan was observed 120 min after the start of the photooxidation procedure, when BT was completely detoxified. Tryptophan content decreased by 33% (Boroff, DasGypta, 1964). The same authors established, in a study of the effect of hydrogen peroxide on BT, that oxidation of 42 moles of methionine residues did not affect toxicity of BT. These data justify dismissal of the assumption that methionine residues are present in the toxophore group of BT.

After treating BT with Koshland reagent, which reacts mainly with tryptophan and, to a lesser extent, cystein, there was a 99% decline in toxicity and 23% decrease in number of tryptophan residues (Boroff, DasGupta, 1966).

Specific modification of arginine residues in BT with 1,2-cyclohexanedione (0.2 M borate buffer, pH 8) inactivated BT-E and BT-A. The solitary polypeptide BT-E chain after modification of arginine residues lost its capacity for lysis by trypsin, whereas serological reactivity of the extracellular BT-A form changed. The obtained data were indicative of involvement of arginine residues in formation of toxophore and serologically reactive centers in BT. It is assumed that proteolysis of the solitary chain of BT-E into two fragments is referable to arginine (DasGupta, 1979).

It must be noted that toxicological experiments on animals are not enough to determine whether a given amino acid is referable to the toxophore center. The ultimate toxic effect, even in the simplest instance, is achieved as a result of several stages: transport of toxin to stricken tissue, binding with specific receptors and actual toxic effect. Direct studies of interaction of toxins with their molecular targets are needed.

TT Binding by Nerve Tissue

The cellular elements of the nervous system are capable of fixing a significant amount of TT (Wasserman, Takaki, 1898), and the brain of toxin-sensitive animals binds more TT than that of resistant animals. It has been established that TT is bound by gangliosides that are in a complex with cerebrosides (Bernheimer, Van Heyningen, 1961; Van Heyningen, 1959, 1963; Van Heyningen, Miller, 1971). Disialoganglioside G_{D_1b} and trisialoganglioside G_{T_1}, whose molecule contains two sialic acid residues, are chemical receptors of TT in nerve tissue. The bond between sialic acid residues is sensitive to neuraminidase (Van Heyningen, 1976). The terminal galactose residue is also important to binding (Van Heyningen, Mellanby, 1973). The data on stoichiometry of interaction indicate that two molecules of sialoganglioside (molecular mass 18,000) are bound with one TT molecule (molecular mass 66,000) (Van Heyningen, Mellanby, 1968). In the absence of protective colloids, the gangliosides themselves inactivate staphylococcus, tetanus and dysentery toxins (North, Doery, 1961), as well as botulinus toxin (Van Heyningen, Mellanby, 1973). It was shown that the binding capacity of the ganglioside and cerebroside complex is largely determined by the quantitative proportion of these components in the complex. Thus, it is 50 times greater in a complex containing 25% gangliosides than complexes with 2 and 50% thereof (Van Heyningen, Mellanby, 1968). Saturation of gangliosides with TT does not protect the bond between sialic acids against the effect of neuraminidase (Bernheimer, Van Heyningen, 1961; Kryzhanovskiy, Sakharova, 1971).

Experiments involving subcellular separation of nerve tissue offered more evidence of the leading role of gangliosides in TT binding. It was found that there is a distinct correlation between amount of gangliosides in the subcellular fraction and its capacity to bind TT (Patel, Rao, 1966). Fractions rich in external neuronal membranes and, consequently, rich in gangliosides--myelin and synaptosomal--bind TT much better than microsome, mitochondrial or synaptic bleb fractions (Mellanby, Whittaker, 1968; Bondarchuk et al., 1973).

The bond between TT and nerve tissue is probably due to physical adsorption. In favor of this hypothesis are data on speed of binding and little dependence thereof on temperature, as well as the fact that gangliosides do not undergo

chemical changes associated with binding (Van Heyningen, 1959a). The bond between TT and nerve tissue is not stable, and it can be readily disrupted by eluting cells with saline (Patel, Rao, 1966b).

A study was made of optical and electric characteristics of a bimolecular ganglioside-lecithin film in order to determine the possible functional significance of TT binding with gangliosides. Measurement of the thickness of the membrane revealed that TT is arranged in one layer on its surface, and the bound TT molecule is opened up. TT binding did not affect the electric characteristics of the membrane. TT itself also failed to undergo appreciable changes; in particular, its capacity to react with antitoxin was retained (Cloves et al., 1972). Experiments with protagon demonstrated that receptor-fixed toxin reacts with antitoxin (Bondarchuk et al., 1973). Since detoxification of TT does not eliminate interaction with gangliosides, while their binding of TT does not impair its reaction with antitoxin, it appears probable that the toxophore, haptophore and antigen determinants are represented by different segments of the TT molecule (Bondarchuk et al., 1973; Kryzhanovsky, 1973; Kryzhanovsky et al., 1975). Recent advances in isolation of fragments, identified as the antigenic (Helting, Zwisler, 1974) and haptophore (Van Heyningen, 1976) determinants of TT serve as experimental proof of the validity of this hypothesis.

Like other agents bound by gangliosides (cholera toxin and wheat grain agglutinin), TT is capable of penetrating through the membranes of peripheral nerve endings (motor and sensory) and migrating within axons into the central nervous system (Erdmann et al., 1975; Stoekel et al., 1975). Preincubation with G_{M1} gangliosides prevented entirely penetration of cholera toxin into axons, whereas G_{T1} ganglioside depressed by 50% access of TT into nerve endings (Stoekel et al., 1977). Although the involvement of gangliosides in toxin capture is terminal, and TT and BT transport over axons into neurons was proven (Habermann, Erdmann, 1978), the pathogenetic significance of TT binding by gangliosides is questionable (Mellanby et al., 1973). It is known that nontoxic fragments of the heavy chain of TT has greater affinity for gangliosides than unadulterated TT. The capacity to be captured by nerve endings and trasported by axons is fully retained by the nontoxic TT fragment (Bizzini et al., 1977).

Recently, a study of TT binding by membranes of murine neuroblastoma cells by the immunofluorescent method revealed (Zimmerman, Piffaretti, 1977) that these cells demonstrate two types of TT binding. One type is demonstrable in cultures of both growing and differentiating cells. Such binding is not associated with functional changes in cells and is eliminated by agents that attack gangliosides (neuraminidase and β-galactosidase). In differentiating cells, TT binding independent of gangliosides was demonstrated, which led to shortening of the cell processes and weakening of their bond with glass, but not cell death. The authors called this type of binding active [effective]. BT is also bound with membranes of nerve endings.

When incubated with a brain homogenate, ^{125}I-labeled BT-A selectively binds with the synaptic fraction. BT is released from this fraction after treatment with triton X-100, sodium dodecylsulfate and neuraminidase (Kitamura, 1976).

Effects of TT and BT on the Central Nervous System

TT strikes inhibitory nerve endings that secrete glycine and γ-aminobutyric acid (GABA). In the presence of tetanus intoxication, stimulation of glycinergic nerve endings does not elicit the usual postsynaptic hyperpolarization potentials in motoneurons (Sverdlov, 1969; Kryzhanovskiy et al., 1971b) and internuncial neurons (Curtis, de Groat, 1968). Since sensitivity of the postsynaptic membrane to glycine is retained (Curtis, De Groat, 1968; Gushchin et al., 1970), depression of postsynaptic responses is attributed to impaired glycine secretion. TT has a similar effect on GABAergic nerve endings. TT depresses postsynaptic inhibition of Purkinje cells in the cerebellum (Curtis et al., 1973) and presynaptic inhibition in the spinal cord (Sverdlov, Alekseyeva, 1965; Sverdlov, Burlakov, 1965; Curtis et al., 1973), and in the latter case depression of depolarization of primary afferents--excitatory postsynaptic potentials (EPSP) in the axo-axonal synapse--occurs concurrently with depressed inhibition of motoneurons (Curtis et al., 1973). In unanesthetized animals, the decrease in primary afferent depolarization is obscured by a strong and rather prolonged potential of dorsal radices, which reflects excitatory processes of cells in the ventral cornu of the gray matter (Lutsenko, 1966; Lutsenko, Kryzhanovskiy, 1973, 1975). At this stage of tetanus intoxication, excitatory processes in motoneurons (Brooks et al., 1957; Sverdlov, 1969) and internuncial neurons (Curtis, de Groat, 1968) are apparently not affected. Depression of spinal (Sherrington, 1906; Brooks et al., 1957; Curtis, 1959; Sverdlov, 1960; Kryzhanovskiy, D'yakonova, 1964; Sverdlov, Burlakov, 1965) and descending (Curtis, 1959; Kryzhanovskiy, Sheykhon, 1968, 1970; Kryzhanovskiy et al., 1971b) inhibition is the only cause of convulsive effect of TT on the spinal cord.

With use of large doses of TT or at the late stages of tetanus intoxication (9th-14th days) there is elimination of monosynaptic reflexes (Sverdlov, 1960). Monosynaptic EPSP recorded intracellularly present an increase in latency and decrease in amplitude (Mikhaylov, Shvarts, 1969).

In the presence of botulinus intoxication, there is much more marked depression of motoneuron excitability than with tetanus. Complete elimination of reflexes of the anterior radices of the spinal cord was observed 72 h after intramuscular injection to cats of $3 \cdot 10^4$ minimum lethal doses (MLD) of BT. Intracellular recording of motoneuron activity revealed a decrease in amplitude of monosynaptic EPSP and decrease in excitability of motoneuron soma as early as 48 h later. Excitability of peripheral nerve fibers was also significantly diminished (Korolev, 1967). A change in these parameters, as well as reduction of input resistance and resting potential of the membrane were found only in phasic motoneurons (Mikhaylov, Mikhaylov, 1975).

Effects of BT and TT on Peripheral Synapses

Symptoms of botulinism--muscular paralysis, visual impairment and impaired intestinal function--are the result of effect of BT on cholinergic synapses (Wright, 1955). Disturbances in cholinergic innervation were reproduced in isolated preparations (Ambache, 1948; Burgen et al., 1949). It was demonstrated in experiments with an isolated nerve-muscle preparation from the guinea pig

that BT depressed discharge of acetylcholine from nerve endings induced by nerve stimulation, and that sensitivity of the pos synaptic membrane to exogenous acetylcholine was retained. There were no changes in the enzyme of acetylcholine synthesis and hydrolysis--cholinacetyl transferase and acetylcholinesterase. Agents with a postsynaptic type of effect (α-tubocurarine, eserine and prostigmine) do not have an appreciable effect on development of botulinic paralysis (Burgen et al., 1949).

Development of botulinus-induced disturbances in myoneural conduction has a number of distinctive features. The time required for them to appear is shortened when the uosage is increased, but only up to a certain limit (Burgen et al., 1949). Activity of nerve endings is of substantial significance to acceleration of damage to the myoneural synapse: stimulation of a nerve at a higher frequency leads to faster depression of synaptic transmission (Hughes, Whaler, 1962; Simpson, 1971).

Myoneural transmission impaired by BT may be restored for some time by means of agents that stimulate acetylcholine secretion. Thus, a volley of pulses at a frequency of 30-100/s lasting 20-60 s alleviated conduction of subsequent test pulses for 1-2 min (Brooks, 1954). Temporary restoration of synaptic conduction was also observed when the concentrations of Ca^{2+} in the incubation medium were doubled (Thesleff, 1960).

BT depressed not only evoked, but spontaneous acetylcholine secretion. A drastic reduction of frequency of miniature potentials of the end-plate (MPEP) was observed (Brooks, 1956; Harris, Miledi, 1971; Spitzer, 1972; Kao et al., 1976; Lund et al., 1976), as well as decrease in MPEP amplitude (Boroff et al., 1974; Kao et al., 1976).

In the presence of botulinus intoxication there is impaired conjugation of presynaptic depolarization with acetylcholine secretion. Depolarization of the membrane of the presynaptic terminal caused by increasing the K^+ concentration to 24 mM was usually associated with 100-fold increase in frequency of MPEP of the muscle fiber. The increase in frequency of MPEP of a preparation infected with BT-D was insignificant under the effect of the same concentration of K^+ (Harris, Miledi, 1971).

The searches made for the primary element in damage to mediator secretion with botulinus intoxication do not enable us to make an unequivocal conclusion. In the presence of chronic botulinus intoxication induced by a small dose of BT, use of agents that either cause migration of Ca^{2+} to the terminal (ionophore A 23 187 and tetraethyl ammonium) or increase in its concentration due to intracellular reserves (guanidine) leads to an increase in frequency of MPEP and amplitude of endplate potential to a normal level, as well as complete restoration of myoneural conduction (Lund et al., 1976). On the basis of these data, the cited authors conclude that the mechanism of isolation of transmitter is not impaired in the presence of botulinus poisoning, but higher than usual concentrations of Ca^{2+} are required to trigger it.

The opposite findings were made in another study (Kao et al., 1976). High doses of BT lowered drastically the frequency and amplitude of MPEP. Use of Ca^{2+} ionophores (A 23 187 and X 537A) failed to elicit an increase in MPEP

frequency. Treatment of BT infected myoneural preparation with karakurt spider [Latnrodectus tredecimguttatus] venom was associated with discharge of the same amount of acetylcholine "quanta" as normally, which was indicative of integrity of all stages preceding exocytosis. In terminals of botulin-infected preparations depleted by spider venom, there was distinct demonstration of an accumulation of synaptic blebs at the site of secretion of transmitter. Hence, it was concluded that BT impairs interaction between synaptic blebs and membrane components involved in exocytosis.

The first convincing evidence of the effect of TT on acetylcholine secretion by nerve endings was obtained from experiments involving injection of TT into the anterior chamber of the rabbit eye (Ambache et al., 1948). TT blocked conduction of excitation in the cholinergic synapse, whereas adrenergic innervation did not undergo any changes. There was a decrease in acetylcholine content of the media of the eye and, to a lesser extent, the iris, although acetylcholinesterase activity did not change. The heightened sensitivity of the pupillary sphincter to carbaminoylcholine was indicative of development of the denervation syndrome. It was concluded that impairment of conduction in cholinergic synapses was due to depressed secretion of acetylcholine from nerve endings.

Use of large doses makes it possible to clearly distinguish between the peripheral and central effects of TT. Gradual impairment of myoneural conduction was observed after intramuscular injection of 1000 MLD to rats, and it was complete after 15 h (Lutsenko, Kasymov, 1963). By this time, TT had only reached the brain and monosynaptic reflexes did not change, while polysynaptic ones were accentuated. Hence, the peripheral block is a very independent phenomenon and does not reflect the effect of TT on central neurons.

In large doses $(25\text{-}250)\cdot10^3$ MLD, TT eliminates the peripheral inhibition induced by GABA secretion. There was no change in sensitivity of postsynaptic membranes to exogenously applied GABA. Later on, TT inhibited synaptic transmission in the exciting synapse as well. Apparently, the specificity of presynaptic effect of TT is not absolute (Kano, Ishikawa, 1972).

Impairment of not only evoked, but spontaneous acetylcholine secretion was demonstrated in myoneural preparations isolated from animals infected with TT. A decrease in MPEP frequency was found in the myoneural synapse of the rat (Kryzhanovskiy et al., 1971a), mouse (Duchen, Tonge, 1973) and goldfish (Mellanby, Thompson, 1972). With chronic tetanus intoxication, there was also a decrease in MPEP of some muscle fibers (Duchen, Tonge, 1973). Potassium depolarization of a nerve ending leads to less than normal increase in MPEP frequency (Duchen, Tonge, 1973; Kryzhanovsky et al., 1978). These data are indicative of impaired coordination [conjugation] of depolarization and secretion, which is also confirmed by less sensitivity of spontaneous secretion to the conjugation factor, Ca^{2+} ions (Kryzhanovsky et al., 1978). The effect of TT on the secretory mechanism is not limited to Ca^{2+}-sensitive processes: elevation of osmotic pressure of the incubation medium elicits less than normal increase in MPEP frequency in an infected preparation (Smirnova, 1976). In spite of the profound damage by TT to the secretory mechanism, some of the regulatory effects remain unaffected by TT. Rhythmic stimulation of a motor nerve, monoamines (Polgar et al., 1972; Kryzhanovsky et al., 1978) and La^{2+} ions (Mellanby, Thompson, 1975) can temporarily increase the MPEP frequency in a preparation infected with TT.

At the present time, the list of mediators whose secretion could be impaired by TT already includes glycine, GABA and acetylcholine. It is assumed that TT affects the secretory mechanism regardless of chemistry of the mediator (Kryzhanovsky, 1973).

Distinctions of Denervation Syndrome Associated With Botulism and Tetanus

Several days after BT and TT impair myoneural transmission, typical signs of denervation appear: fibrillation of muscle fibers that is not removed by severing the motor nerve (Prabhu, Oester, 1962; Prabhu et al., 1962; Thesleff, 1960), heightened sensitivity to acetylcholine (Thesleff, 1960; Joseffson, Thesleff, 1961; Prabhu et al., 1962; Duchen, Tonge, 1973; Bray, Harris, 1975) and later on muscular atrophy (Duchen, 1969; Duchen, Tonge, 1973).

The decrease in incidence of MPEP in the case of botulin poisoning is associated with changes in distribution of MPEP according to amplitude, which are inherent in denervation. The Gaussian distribution changes to asymmetrical, with a disproportionately large number of low-amplitude MPEP (Harris, Miledi, 1971; Bray, Harris, 1975). Rhythmic stimulation of the nerve or potassium depolarization elicits higher amplitude MPEP with almost normal statistical distribution of amplitudes (Harris, Miledi, 1971; Duchen, Tonge, 1973; Bray, Harris, 1975). Demonstration of two classes of MPEP in the same synapse is of considerable interest to analysis of secretory mechanisms, since it is indicative of differences in acetylcholine quanta. The assumed contribution of Schwann cells to generation of low-amplitude MPEP in the case of anatomical denervation appears unlikely, since there is no degeneration of endings in the presence of botulism and tetanus (Duchen, Tonge, 1973; Bray, Harris, 1975). Perhaps, BT impairs the mechanism of filling or emptying of the vesicles.

The considerable duration of toxin-induced denervation and possibility of controlling the degree of impairment of myoneural transmission make it possible to conduct experimental studies of the basically important question of nature of trophic influences of the nerve on the muscle. In the case of tetanus, muscular sensitivity to acetylcholine occurs at the stage when the level of spontaneous secretion of acetylcholine differs little from normal (Duchen, Tonge, 1973). In the case of botulin poisoning, heightened sensitivity to acetylcholine appears in muscle fibers with virtually intact synaptic innervation and disappears even before myoneural transmission is restored (Bray, Harris, 1975). These facts are interpreted by the cited authors as evidence of existence of a special factor, other than mediator, that regulates metabolic and membrane processes in the muscle, i.e., a specifically trophic factor.

It is known that, at the late stages of intoxication, BT and TT depress axon current in the frog's peripheral nerve (Mikhaylov, Chekhovskaya, 1969). In the case of botulinism in rats, there was no depression of rapid axonal transport of proteins, but the amount of radioactive material transmitted decreased by 75%. There was accumulation of radioactive material in nerve endings and adjacent part of a BT-infected axon, probably due to impaired discharge thereof by nerve endings (Bray, Harris, 1975). With botulinus poisoning of the frog's slow muscle, the increase in sensitivity to acetylcholine was not associated with appearance of capacity to generate action potentials, as in the case of anatomical denervation. This warrants the belief

that BT depresses only secretion of trophogen, which depresses synthesis of cholinoreceptor, but not the trophogen that regulates appearance of Na^+ ionophore (Miledi, Spitzer, 1974).

Molecular Mechanisms of Effects of BT and TT

Introductory remarks: It was shown above that BT attacks primarily the peripheral cholinergic synapses, whereas TT attacks amino-acidergic ones in the central nervous system. The selectivity of effects of the toxins on synapses of a specific chemical type prompted researchers to investigate, first of all, the effects of BT and TT on specialized metabolic reactions of the corresponding mediators and processes of their transport through nerve ending membranes.

Effect on cholinergic processes: The first stage of investigation of molecular mechanisms of effects of a chemical agent consists of an attempt to reproduce its effects in vitro, in simpler systems. It was established that incubation of sections of the cerebral cortex or neuron cultures in media containing BT and TT lead to depression after 2-4 h of synthesis and release of acetylcholine into the incubation medium (Molenaar, Pollak, 1970; Gundersen, Howard, 1978; Bigalke et al., 1978). However, these facts cannot be interpreted as proof of the toxin's direct intervention in acetylation of choline or damage to the mechanism of release of acetylcholine. Significant depression was demonstrated in highly affine capture of choline in synaptosomes isolated from BT-infected sections. Thus, both depression of synthesis and of acetylcholine discharge by potassium depolarization of infected sections was apparently merely the consequence of initial impairment of choline transport (Gundersen, Howard, 1978).

Different results were obtained with the use of large doses of BT on synaptosomes. After incubation of synaptosomes with BT, discharge of acetylcholine induced by potassium depolarization decreased by 80% (!). Capture of choline from the medium, acetylcholine synthesis in synaptosomes, nature of distribution of acetylcholine in cytoplasm and vesicles did not undergo appreciable changes in the presence of BT. Thus, in these experiments BT impaired only the mechanism of acetylcholine secretion (Wonnakot, Marchbanks, 1976). A significant increase in Ca^{2+} concentration in the medium virtually eliminated differences in amounts of acetylcholine released by K^+ depolarization from BT-treated and intact synaptosomes (Wonnacot et al., 1978)

Impairment of depolarization-induced discharge of acetylcholine from nerve endings could be the result of blocked access of Ca^{2+} in the terminal; however, the facts contradict this hypothesis: BT does not affect accumulation of Ca^{2+} by synaptosomes (Drachman, Fanburg, 1969; Wonnacot et al., 1978).

Effect on amino acid metabolism: There are contradictory data on amino acid content of spinal cord tissue in the presence of tetanus. In the case of generalized tetanus in cats, the spinal cord showed an increase in aspartic acid content (Johnston et al., 1969). With local tetanus, there was no change in GABA content of the stricken half of the brain, but there was a reliable decrease in glycine (Semba, Kano, 1969). In another study, also conducted on cats, no changes were demonstrable in levels of glycine, GAPA, glutamate or asparate in the presence of either local or generalized tetanus (Fedinec, Shank, 1971).

It is known that the effects of convulsants, which intervene in GABA metabolism, show a correlation to level of activity of glutamate decarboxylase synthesized by GABA, rather than GABA content of the brain. Studies of glutamate decarboxylase activity in an unpurified fraction of synaptosome isolated from the spinal cord of rats with local tetanus failed to demonstrate depression of this enzyme's activity. Consequently, the impaired release of GABA from nerve endings treated with TT cannot be attributed to impaired synthesis thereof (Lutsenko et al., 1976).

The data pertaining to release of mediators from sections of the brain and synaptosomes isolated from toxin-treated nerve tissue conform well with physiological evidence of impairment of secretory processes in the same tissues. Potassium depolarization of sections of the striate body and substantia nigra treated in vivo with TT was associated with less than normal release of ^{3}H-GABA and ^{3}H-dopamine. There was no change in accretion of radioactive mediators by sections of toxin-treated structures (Collingbridge et al., 1979).

At the preagonal stage of generalized tetanus, glutamate and GABA content of synaptosomes from the spinal cord and medulla oblongata was 50% increased, whereas the capacity to release amino acids in response to electric stimulation was diminished. Synaptosomes released less glycine, GABA and aspartic acid with electrostimulation than intact synaptosomes. TT added directly to the incubation medium had no effect on synaptosome function (Osborne, Bradford, 1973).

In vitro, BT depressed accretion by synaptosomes of ^{3}H-norepinephrine and particularly ^{14}C-glycine, it increased release of mediators into medium with normal and high K^{+} content (Wonnacot, Marchbanks. 1976). Accumulation of GABA by synaptosomes isolated from BT-treated sections did not differ from the control, whereas highly affine capture of choline was partially depressed (Gundersen, Howard, 1978).

Effect of TT on oxidative phosphorylation in mitochondria: The mitochondria control Ca^{2+} level that triggers the secretory process in a nerve ending, so that it is important to investigate the effect of TT on mitochondria in order to comprehend the mechanism of toxin-induced impairment of secretion. It was demonstrated that unpurified TT disrupts respiration and phosphorylation in mitochondria at the NAD-dependent stage (Patel, Rao, 1966a). Like inactivators ["disconnectors"], unpurified TT intensified significantly the spontaneous release of acetylcholine from motor nerve endings with subsequent impairment of myoneural transmission due to depletion of stock of mediator available for release in the terminal (Parsons et al., 1963). As mentioned above, purified TT depressed both spontaneous and evoked secretion of acetylcholine, i.e., its effect on mechanisms of mediator secretion is different from the effects of inactivators of oxidative phosphorylation.

In experiments with mitochondria isolated from the tissue that is the most sensitive to TT (spinal cord), purified TT was used. It was found that highly purified TT, in doses of 500 to 100,000 MLD for mice, had no effect on energy processes. Mitochondria isolated from TT-treated segments of the spinal cord did not differ from intact ones in magnitude of P/O ratio, although the parameters themselves were higher (Sakharova et al., 1975; Lutsenko et al., 1975).

Studies of the effect of TT on respiration, creatine phosphate production, Na^+ and K^+ content in guinea pig brain sections at rest and with electric stimulation of sections failed to demonstrate changes in metabolic responses in the presence of TT (Evans, McIlwain, 1967).

Effect on transport of monovalent cations: Impairment of electrosecretory conjugation in nerve endings may be due not only to toxin-caused damage to the secretory mechanism, but interference in membrane electrogenesis.

TT and BT are usually considered to be specific toxic agents for expressly the secretory mechanism; however, recent evidence of electrogenic effect of TT and BT on membranes cause us to question the validity of such interpretation. Ramos et al. (1979), who used the lipophilic organic cation, tetraphenyl phosphonium, as a probe for membrane potential, demonstrated that TT is capable of inducing both hyperpolarization and depolarization of membranes of synaptosomes isolated from the guinea pig cerebral cortex. Inactivation of TT with antitoxin or by boiling eliminated its electrogenic effects. The membrane potential of mitochondria does not change in the presence of TT, which is consistent with data concerning mitochondrial resistance to the effects of TT (Lutsenko et al., 1975).

It is known that the membrane potential of neurons and their processes depends on active and passive transport of monovalent cations and integrity of the membrane. In vitro, TT does not affect Na^+, K^+-ATPase activity--the enzyme that effects active transport of monovalent cations; however, in the presence of tetanus, NA^+, K^+-ATPase activity of nerve endings in the rat spinal cord increased (Kryzhanovskiy et al., 1974).

In the presence of botulism, changes in membrane electrogenesis were also noted in the neuron proper (Mikhaylov, Mikhaylov, 1975). In the opinion of the cited authors, persistent membrane depolarization and other changes in electric activity of the neuron under the influence of BT are attributable to modification of Na^+ channels, which leads to elimination of Na^+ inactivation.

Effect of TT on protein synthesis: When TT was incubated with synaptosomes a 60% increase in protein synthesis was demonstrated (Kryzhanovskiy et al., 1975b). Perhaps, the changes in protein composition of synaptic membranes in the presence of tetanus are also related to synthesis of new proteins. Electrophoresis in polyacrylamide gel of the protein fraction extracted from synaptic membranes with detergent revealed an increase in the peak of slowly migrating proteins. Immunochemical studies of antigenic composition of cell organelles using the complement-fixation reaction revealed that a new antigen is demonstrable in nerve ending membranes in the presence of tetanus (Kryzhanovskiy et al., 1975).

Effect of TT on function of contractile proteins: It is assumed that release of mediators from nerve endings occurs as a result of interaction of synaptic membrane actin with synaptic vesicle myosin. In accordance with the hypothesis of involvement of contractile proteins in mediator exocytosis and selective attack of secretory mechanisms by TT, it was demonstrated that actin stimulates escape of ^{14}C-glutamate (Puszkin, Kochwa, 1974) and endogenous norepinephrine from vesicles into the incubation medium, while TT eliminates the

actin-induced release of vesicular norepinephrine (Kryzhanovskiy et al., 1980). In vitro formation of an actin-myosin complex--the superprecipitation reaction--is also impaired by TT. The above-described effects of TT have no pathogenetic significance. TT does not affect adrenergic synaptic transmission or muscular contractions in animals (Ambache et al., 1948).

Conclusion

In this survey, we concentrated mainly on analysis of mechanisms of toxin-induced impairment of mediator (GABA, glycine and acetylcholine) secretion, since it is expressly the damage to synaptic function that determines the symptomatology of tetanus and botulism. The submitted data are indicative of definite advances in the study of the mechanisms of effects of two of the most potent toxins known at the present time. In the case of botulinism, some specifically presynaptic processes are apparently unaffected: storage of acetylcholine in vesicles, migration of Ca^{2+} to the terminal and actual mechanism of acetylcholine secretion. Botulinism apparently amounts to decline in sensitivity of the secretory mechanism to Ca^{2+}. All factors that increase the intracellular concentration of Ca^{2+} restore the impaired secretory function of the nerve ending.

The target of TT is secretion of neutral amino acids by nerve endings, which have an inhibitory effect on central neurons. The mechanism of impairment of acetylcholine secretion in peripheral synapses by BT and large doses of TT is probably the same. The hypothesis can be expounded that the presynaptic effect of both toxins is a special instance of their more general membranotropic effect. In addition to depression of Ca^{2+}-dependent processes (secretion of mediators and axoplasmic current, in the presence of tetanus and botulinism there is a change in processes that are determined by or depend on the transmembrane Na^{+} and K^{+} gradient (action and resting potentials, highly affine capture of mediators, osmotic sensitivity). Thus, impaired secretion of acetylcholine could be the result of depression of Na^{+}-dependent choline capture, since the capture of choline controls, to some extent, acetylcholine synthesis, and there is secretion of mainly de novo synthesized mediator.

Aside from their common clostridial origin, TT and BT are also similar in the physicochemical characteristics of their molecules: both toxins are acid proteins with molecular mass of ~150,000 dalton, which have very similar structure. Both toxins attack the same membrane processes, which is indicative of some similarity of toxic groups.

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MODERN BIOTECHNOLOGY: FRONTIERS AND FUTURE

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[Editorial by V. M. Zhdanov and T. I. Tikhonenko]

[Text] The scientific and technological revolution, which is taking place before our eyes and important for the close ties between basic and applied scientific research, on the one hand, science and industry, on the other, could not help but involve the biological sciences. At the present time, an important process is taking place in biology: the distance between basic discoveries and practical applications thereof is being overcome rapidly. The results of this process are obvious: in the last decade, the microbiological industry has emerged, biological catalysts are used extensively in industry, the achievements of genetics have been recognized in breeding antibiotic producers and other useful microorganisms, biological methods are being used extensively in the control of agricultural pests and many others. But it was the most vividly manifested, as stressed in the decree of the CC CPSU and USSR Council of Ministers published on 30 July 1981, concerning further development of physicochemical biology and biotechnology with use of advances thereof in medicine, agriculture and industry, by the formation of a new scientific applied sector, biotechnology, which is the organic blend of technical biochemistry, microbiology, genetic engineering, combined with the use of animal and plant cell cultures and immobilized enzymes. It includes both age-old processes (cheese making, bread baking and wine making) and new technological processes (microbiological production of fertilizers, vitamins, antibiotics and the latest achievements of gene engineering). In the postwar years, biotechnology underwent major development in our country; we have a microbiological industry, which has and continues to contribute much to agriculture and public health. Suffice it to mention agricultural fertilizers, without which it would be inconceivable to fulfill the food program worked out by the CC CPSU, as well as antibiotics, without which progress of medicine and public health care is impossible.

In the "Main Directions of Economic and Social Development in 1981-1985 and the Period up to 1990," the 26th CPSU Congress outlined implementation in the microbiological industry of measures for accelerated development of production based on microbiological synthesis, so that there would be a 1.8-1.9-fold increase in output of products. These plans are also being coordinated with tasks put to medical science, such as learning the mechanisms of

physiological, biochemical, genetic and immunological vital processes, improvement of methods for the prevention, detection and treatment of the most widespread diseases, development of new drugs, products and medical equipment. In the area of public health, there are plans for construction of new polyclinics and hospitals, furnishing them with new medical equipment, production of new and effective drugs, assuring prompt and high-quality medical services for each citizen of our country.

Development of new biotechnology and its basis--gene engineering--will be quite important to the solution of these problems. Gene, or genetic, engineering appeared as a special direction on the boundary of molecular biology, microbiology and virology less than 10 years ago, and immediately defined its subject, its methods and objectives of investigation. Genes--segments of DNA coding synthesis of different proteins, as well as DNA sequences regulating gene activity, became the objects of its studies. In many instances, one had to deal with copies, rather than genes themselves, messenger RNA that directly coded protein synthesis on ribosomes. In such cases, it was necessary to prepare genes, using RNA as the matrix for synthesis of complementary DNA chains. Finally, direct chemical synthesis of genes is possible, if their nucleotide sequence or amino acid sequence in proteins coded by these genes is known. Viruses are used to transfer genes to a heterogeneous system and, even more often, virus-like genetic structures--plasmids or episomes, which are extrachromosomal genetic factors. They play an important part in bacteria and other unicellular organisms (for example, yeast). Corynebacterium diphtheriae, for example, is incapable of producing exotoxin, this property is imparted to it by bacterial virus--temperate bacteriophage, which is a parasite in C. diphtheriae. Another example is bacterial resistance to antibiotics: it is usually related to intracellular plasmids that bear the genes of enzymes that break down or modify antibiotics. Viruses and plasmids that transfer genetic information are designated as vectors. Obviously, the appropriate viruses or their DNA can be vectors for eukaryote cells, including those of animals and man. At the present time, efforts are also being made to use cytoplasmic elements of cells of the mitochondrial type, at least mitochondrial DNA, as vectors.

An assortment of highly specific enzymes--restrictases and ligases--is used to insert a gene that interests us into plasmid or viral DNA. Restrictases are used to cut DNA molecules in strictly specific sites. For example, EcoR1 restrictase cuts ring molecules of plasmid pBR 322, the DNA of OB40 virus or hepatitis B in one site, changing the circular molecules of these DNA into linear ones. The enzyme, ligase, can be used to join the ends of different molecules, forming recombinant (hybrid) circular molecules of DNA of plasmid pBR 322 and OB40, or DNA of plasmid pBR 322 and hepatitis B. In a number of instances, such recombinant molecules can replicate in appropriate host cells. Thus, the recombinant molecule of plasmid pBR 322 and OB40 virus replicates in E. coli cells (host of plasmid pBR 322) and primate kidneys (host of OB40 virus).

If the initial material is molecules of messenger RNA (for example, for insulin, globin, interferon) or viral RNA (for example, the genes of influenza virus--fragments of RNA or an entire RNA-containing genome of poliomyelitis virus), for insertion in viral or plasmid DNA one must first perform template synthesis of single-stranded DNA complementary to the RNA template, and then synthesize

the second DNA strand. For this purpose, one uses the enzyme, reverse transcriptase (revertase), which is contained in oncoviral virions (avian myeloblastosis virus is generally used), as well as bacterial enzymes of DNA synthesis. In such cases, "sticky ends"--sequences of oligonucleotides that are complementary to one another (oligodT and oligodA, oligodG and oligodC)--are added to the molecule of the vector (plasmids, viruses) of the gene synthesized on the RNA template in order to thus form a circular recombinant molecule. Revertase is also used in other instances in biotechnology, which we shall discuss below.

All of the foregoing is referable to the first stage of gene engineering procedures for producing recombinant DNA molecules containing genes of interest to us. Addition of such recombinant DNA, which were formed on the basis of plasmid or viral DNA, makes it possible to reproduce the genes of interest in considerable amounts provided, of course, that the recombinant molecule is viable and transfection (infection of cell with recombinant DNA) was successful. However, this is only the end of the first phase of work, since the above-mentioned procedures do not usually produce the genes of interest in a functionally active state, while our objective is expression thereof, which is manifested by synthesis of proper proteins that are coded by these genes. The absence of expression can be due to different causes--absence of promoter or presence of a mild promoter that effects expression of a given gene, "frame shift," i.e., elimination or surplus of 1-2 nucleotides, as a result of which there is change in the triplets that code specific amino acid residues in the polypeptide chain, and many other causes. It would not be superfluous to also recall that there is a difference in protein synthesis in prokaryote (bacterial) and eukaryote cells with regard to both the initiating codon and corresponding initial amino acid residue (formyl methionine and methionine, respectively), as well as minor transfer RNA, which limit the rate of synthesis; moreover, glycosylation of polypeptide chains is important to many proteins, and this process is different in prokaryotes and eukaryotes.

Still, the most significant obstacle to synthesis of protein in bacterial cells on a template of cloned human and animal genes is the basic difference in structure and function of prokaryote and eukaryote genomes. As we know, most animal genes are mosaic in nature and consist of alternately silent (intron) and expressed (exon) parts. All of these sites are transcribed in the form of a primary transcript, from which intron sequences (so-called splicing) are removed thereafter when mature mRNA is formed. Thus, in the globin gene of some systems there are up to five intron sections. At the same time, mRNA and its gene are colinear in the bacterial cell, i.e., the gene is transcribed completely in mRNA, with the exception, of course, of its regulatory segment. For this reason, for example, transfer of an intact human globin gene into a bacterial cell would not lead to formation of this protein. In this case, protein synthesis would be blocked in bacterial ribosomes, most probably in the very first intron sequence. Moreover, even if the intron sequences are translated into some amino acid sequence, in spite of our expectation, the presence of unusual polypeptide sections corresponding to introns would alter radically the properties of the protein molecule. For this reason, it is necessary to remove the intron sequences for synthesis of most animal and human proteins in bacterial cells. In practice, this is achieved by submitting the preparation of individual mRNA of eukaryote origin to reverse transcription in

vitro by means of retroviral revertase, as described above. There is another possible way: expression of animal and human genes in protozoan unicellular eukaryotes of the yeast type with the expectation of correct splicing. In this respect, the first encouraging results have been obtained, but the use of yeast as objects of microbiological synthesis of animal and human proteins requires further investigation. Another possible means is expression of recombinant DNA in cultures of animal cells, mainly in suspension cultures of lymphoblastoid cells that can be cultivated in fermenters with capacity of up to 3-5 m^3. This method could be used extensively, if we succeed in lowering appreciably the cost of the process of cultivating such cells, since the cost of culture media is very high.

When researchers turn from prokaryote to eukaryote systems, they also encounter other, more secondary difficulties of obtaining adequate expression, but we shall not dwell on them here. At any rate, as can be seen from the foregoing and as shown by research of recent years, these difficulties can be overcome in both the use of prokaryote cells and resort to protozoan eukaryote cells, for example, yeast or suspension cultures of animal cells.

The third, deciding stage of gene engineering is development of technology, which must be efficient and economical. In other words, bacterial or yeast cultures containing recombinant molecules with the genes of interest to us must assure expression of these genes, i.e., synthesis of the appropriate proteins in quantities that would be profitable, as compared to the usual technology for production of the same proteins (antigens).

The above, rather simplified description of procedures of gene engineering shows how difficult are the tasks put to specialists working in this field. Nevertheless, the increased sophistication of Soviet science, existence of highly qualified personnel and scientific-technical equipment make it possible to perform these tasks well. The achievements made in different directions were recently demonstrated (December 1981) at a session of the USSR Academy of Sciences (paper delivered by Academician Yu. A. Ovchinnikov, vice-president of the USSR Academy of Sciences, and discussion thereof). There is also some serious work being done at the scientific institutions of the USSR Academy of Medical Sciences and USSR Ministry of Health. The situation that has developed in our country can be described in one phrase: "Biotechnology: on the Eve of Taking Off" (PRAVDA, 3 Jan 1982).

Use of gene engineering methods to produce vaccines against viral hepatitis (B and A), influenza, tick-borne encephalitis, adenoviral and other viral infections is a task that must be performed in the foreseeable future for application to virology. Hepatitis viruses A and B submit poorly to cultivation outside the human body, while recovery of antigen of hepatitis virus B from human blood is far from being the best solution to the problem, so that only gene engineering can provide a radical solution. Production of influenza vaccines by means of gene engineering would make it possible to abandon the use of a valuable food, hen eggs. In addition, the use of gene engineering methods makes it possible to obtain needed proteins (for example, hemagglutinin and neuraminidase of influenza viruses), rather than whole virions, and this would solve two problems at the same time: we would get rid of inactive proteins and rule out the risk of infection by incompletely inactivated virus,

which is far from unimportant for vaccines against serious viral infections (tick-borne encephalitis, hepatitis B, rabies, etc.). In the longer term, we expect that there will be microbiological synthesis of antigenic determinants of viral proteins, which will make it possible to produce polyvalent vaccines. Nor is there any need to prove the importance of using gene engineering to produce human interferon, which has demonstrated its efficacy, not only against viral infections, but oncological diseases.

We can express our confidence that the joint efforts of the leading virological and microbiological scientific institutions in performing the tasks put by the Party and government in the area of biotechnology, a complex [combined] approach and good coordination of scientific research, rapid issue of scientific achievements in technology and industry will enrich Soviet public health with new and effective means of preventing and treating viral infections.

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PRODUCTION OF ANTIBODIES TO FRACTION 1 OF YERSINIA PESTIS IN INTRAGASTRICALLY INFECTED COMMON VOLES

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[Article by A. Ye. Suvorova, A. A. Vartanyan, M. T. Shekhikyan, A. G. Mnatsakanyan and V. V. Oganesyan, Armenian Plague-Control Station]

[Text] It was established that, in common voles, antibody production occurs later after intragastric infection than hypodermic. The antibody titer did not reach the analogous parameter of voles infected subcutaneously at any of the tested times.

Key words: antibody production, common vole, plague.

Rodent--flea--rodent is the principal route of circulation of the pathogen of plague. However, under the conditions prevailing in a Transcaucasian high-altitude site, several researchers succeeded in tracking alimentary infection of voles with the pathogen of plague as a result of cannibalism [4, 8]. For this reason, it was of some interest to investigate production of antibodies to fraction 1 of Yersinia pestis in common voles after infecting them by mouth. It was necessary to take into consideration the fact that, in their natural habitat, plague-ridden animals could be consumed both after a prolonged period of starvation and without prior starvation of voles, whereas the activity of gastrointestinal enzymes would differ under these conditions. It is known that starvation of guinea pigs and baby rabbits leads to a marked increase in intestinal alkaline phosphatase content within the first hours, after which its level drops and holds at relatively low values for 15-20 h [2].

In view of the fact that alkaline phosphatase of the intestine plays some part in the pathogenesis of intestinal infections [6, 7] and expression of pathogenicity of El Tor and NAG vibrios [3], we tried to determine its influence on production of antibodies to factor 1 of Y. pestis after infection by mouth. We activated intestinal alkaline phosphatase in the voles by means of hypodermic injection of a solution of magnesium sulfate, since experiments on guinea pigs and baby rabbits proved that magnesium affects the level of this enzyme in the intestine of experimental animals [1].

Material and Methods

Preliminary experiments to determine changes in alkaline phosphatase during starvation, effect of hypodermic injection of magnesium sulfate on change in this enzyme in the vole intestine, as well as the main experiments dealing with antibody production after infection with the pathogen of plague by mouth, as compared to antibody production after hypodermic infection, were conducted on young voles of both sexes trapped in a region of Armenian SSR that was not epizootic for plague.

The voles caught for these experiments were quarantined for 15 days, and all animals that died within this time were tested bacteriologically and serologically for plague. Before starting the experiments six animals were sacrificed and tested for plague. The results were negative.

We made quantitative assays of alkaline phosphatase in 56 common voles submitted to starvation. At the start of starvation, half the animals were given hypodermic injections of 0.2 mℓ 6.2% magnesium sulfate solution to activate this enzyme in the intestine [1]. The optimum dosage of this compound had been determined in preliminary tests on 20 voles.

We assayed alkaline phosphatase by the method of Fomina [6] 30 min, 1.5-2, 5-7 and 18-20 h after starting to withhold feed. The obtained data revealed that an increase in this enzyme occurred 30 min after discontinuing feeding. Animals given magnesium sulfate at this time showed a considerably more marked increase in the enzyme that in animals who did not receive it (11,000 units/g, versus 7000 units/g). After 2 h we observed a significant decline of phosphatase in both groups of animals; for the next 4 h the enzyme content held at the same level with subsequent slow decline.

Thus, administration of magnesium sulfate activated production of alkaline phosphatase in common voles. The highest level of this enzyme was noted 30 min from the start of starvation and injection of magnesium, and this was taken into consideration when the voles were infected with Y. pestis.

We used two methods of infection in the main experiment: intragastric, which simulated infection by natural penetration of the pathogen through cannibalism, and hypodermic, which is similar to infection through the bite of a vector.

We used Yersinia pestis strain 2048 to infect the voles; it was isolated from Ceratophyllus caspius fleas in Gukasyanskiy Rayon of Armenian SSR in 1975. The strains had the typical features of the vole variant; the HIR [hemagglutination inhibition reaction] with antibody diagnosticum showed 0.02 μg factor 1 per 100,000 microbial bodies. The LD_{50} for white mice constituted 25,000 microbial bodies.

Before infecting them, the voles were separated into three groups: the first group (78 voles) was deprived of feed 30 min before infection, at which time magnesium sulfate was given, i.e., this group was infected against a background of maximum alkaline phosphatase content in the intestine. This group of voles was infected with Y. pestis in a dosage of 200 million microbial bodies in a volume of 0.2 mℓ, intragastrically, by means of a syringe with a U-shaped curved

needle, to the end of which an olive-shaped millet-grain sized tip was soldered. The second group (60 voles) was also infected intragastrically, but without prior abstention from feed and magnesium sulfate, i.e., in the presence of the usual alkaline phosphatase level in the intestine. The third group (50 voles) was infected by hypodermic injection in a dosage of 50,000 microbial bodies in a volume of 0.2 mℓ.

The voles that died 1 day after infection were not examined. Starting with the second day, all of the animals that died were submitted to bacteriological and serological examination. We took the same number of surviving animals from each group, but at least 4, sacrificed them and examined them 15, 30, 45, 60 and 90 days after infection.

Washings from the thoracic cavity [5] served as material for serological tests. The serological examination was conducted in accordance with the "Instructions for Use of Serological Methods...," Saratov (1974). The bacteriological examination was made by preparing impressions of viscera, blood and lymph nodes on agar film. The results were submitted to statistical processing according to Ashmarin and Vorob'yev [3].

Results and Discussion

In the first 7 postinfection days, deaths constituted 19.7±4.2% in the first group, 20.0±5.0% in the second and 25.0±6.0% in the third group of voles. In virtually all cases, the death of animals at this time was associated with bacteriological confirmation, with negative serological reactions.

Three of the eight voles in the first group that died after the 7th postinfection day contained Y. pestis; positive HIR (indirect hemagglutination reaction) was demonstrated in one of these voles and only incomplete antibodies--ANR (antigen neutralization reaction)--in the other two.

In the second group of voles, half of the six animals that died presented a positive bacteriological response, there was the same number of animals with positive HIR and ANR and five with only ANR. In one of the four voles infected subcutaneously we succeeded in isolating Y. pestis; two presented incomplete antibodies and the positive bacteriological test results coincided with the serological ones. Titers of complete antibodies constituted 1:40-1:160 in animals that died after the 7th postinfection day; incomplete antibody titers fluctuated over a wide range, from 1:40 to 1:640, but higher titers were not demonstrated in any of the animals.

Examination of surviving voles 15 days after infection showed negative bacteriological tests. The number of animals with seropositive reactions differed in the different groups. Thus, in the first group, the ANR was positive in only one out of four sacrificed animals, in the second group in three and in the third all of the animals reacted in the ANR.

There were rather substantial differences in serological reactions 30 days after infection. In the first group of animals infected intragastrically in the presence of an elevated alkaline phosphatase level in the intestine, 2 out of 4 sacrificed animals showed an HIR reaction and three ANR, the

geometric mean titers being 1:140 and 1:160, respectively; in the second group, only one vole presented a positive serological response; in the third group, which was infected subcutaneously, all of the animals reacted in both serological tests, and the reaction titers were considerably higher in this group than in the first and second.

The first group of voles presented the maximum amount of incomplete antibodies 45 days after infection. The titers of complete antibodies continued to rise and reached a maximum only by the 60th postinfection day. By this time, the quantity of incomplete antibodies diminished. At subsequent observation times, there was a decrease in amount of both complete and incomplete antibodies.

On the 45th, 60th and 90th postinfection days, the second group of voles presented slow increase in antibodies to factor 1 of Y. pestis. The titers of serological reactions were lower at all tested times in this group of animals than in the first or third.

The results of our studies lead us to the following conclusions: the dynamics of production of antibodies to factor 1 of Yersinia pestis in common voles infected by mouth, with the usual level of intestinal alkaline phosphatase, differed from antibody production in voles with elevated enzyme content at the time of infection, as well as from the analogous parameter in voles infected subcutaneously; the maximum antibody level was demonstrated later in voles infected by mouth than in those infected hypodermically. Maximum titer of incomplete antibodies in the group of animals infected at the time of maximum phosphatase content of the intestine was noted on the 45th postinfection day and maximum complete antibody titer on the 60th day. Antibodies continued to rise up to the 90th postinfection day (duration of observation period) in the group infected intragastrically with the usual amount of alkaline phosphatase. In voles infected subcutaneously, maximum antibodies were demonstrated 30 days after infection; the antibody levels in voles infected by mouth never reached the levels demonstrated in voles infected subcutaneously.

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CLINICAL PROBLEMS OF GENETICS IN NEXT FEW YEARS

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[Article by Ye. F. Davidenkova, academic group of Prof Ye. F. Davidenkova, corresponding member of the USSR Academy of Medical Sciences, Leningrad]

[Text] Genetics is experiencing a period of intensive introduction to the national economy and public health care. The achievements of different branches of molecular genetics, molecular biology, gene engineering, biochemical and medical genetics are offering new opportunities in diagnostics, prevention and therapy of hereditary diseases of man. One of the most important achievements of the last 5-year period was the creation of the theoretical and experimental basis for development of industrial microbiological technology for the production of biologically active polypeptides, which provide needed components for treatment of widespread diseases--diabetes, viral and bacterial infections, malignant neoplasms, neurospychiatric diseases and others. The expected therapeutic effects of these products make it imperative for clinicians to be informed about these advances.

In the last two decades, methods were developed for isolating individual mRNA (V. S. Geytskhoki); chemical (M. N. Kolosov; Crick) and biological synthesis of DNA has been effected, and transfer of information from RNA to DNA--reverse transcription (Temin)--was discovered. The discovery of a new class of enzymes--restriction endonucleases (V. I. Tanyashin)--and development of methods of restriction analysis of DNA (A. A. Bayev) made it possible to gain new information about the structure and function of the eukaryote genome. The most impressive event was discovery of the fact that a structural segment of the gene previously believed to be inseparable was found to be severed by a greater or lesser quantity of insertions (introns) that are not demonstrable in mature mRNA. For example, 50 such inserts were found for collagen (Prockop). Excision and subsequent splicing of significant sequences (exons) in the course of maturation of heterogeneous nuclear RNA lead to creation of mature mRNA (Crick).

In order to acquaint the reader with the theoretical aspects that led to development of bacterial methods of synthesis of polypeptides of human origin, we must discuss some categories of gene engineering and, first of all, sources of isolated genetic material. We refer to segments of DNA, synthesis of which can be effected chemically from dinucleotides and trinucleotides, with recovery of

a product that codes 30-40 amino acid sequences. Synthesis of DNA on an mRNA template by means of reverse transcriptase is another variant of recovery of genetic material. This method is prefereable, since it permits recovery of genes without intron regions, which is necessary for correct expression of these sites in the bacterial cell (T. I. Tikhonenko). In addition to artificially produced molecules, one can also use native DNA isolated from biological systems to reconstruct combinant DNA.

A wide assortment of enzymes and, in particular, the class of restriction endonucleases [restrictases] are used to produce recombinant DNA molecules consisting of the coding part of the gene, working sequences (promoters, operators, terminators, etc.) and vectors (plasmids, viral genomes). Restriction endonucleases can cut DNA molecules at a strictly specific place on the boundary of concrete sequences of nucleotides, while the obtained and selected molecular fragments are spliced with ligases, for example, DNA ligase of E. coli. The obtained recombinant molecules containing structural parts of genes in plasmids or viruses are submitted to cloning (selection) and amplification (reproduction). Further expression of these recombinant molecules, which contain a concrete structural gene, in the bacterial cell leads to creation of effective producers of antibiotics, enzymes, amino acids and proteins with specific biological activity.

Even now, several firms in the United States are involved with commercial use of advances of gene engineering. In our country, as a result of implementation of several programs, in particular the "Revertase" project under the supervision of V. A. Engel'gardt and with wide cooperation among CEMA nations and along CEMA lines, gene engineering studies have been organized, the globin gene was synthesized, and the training of molecular biologists has been set up since they will have to solve problems of introducing gene engineering technology to industry (L. L. Kiselev and V. A. Engel'gardt).

From the clinical point of view, the most important achievement of gene engineering is development of strains that produce enzymes, hormones, neuropeptides of human origin and, in particular, insulin, somatostatin and interferon. Hence the contribution of gene engineering is understandable, as being one of the branches of genetics, as well as genetics of microorganisms, to development of agents for treatment of diabetes, dwarfism of pituitary origin and acute viral infections (interferon). In addition, as it became known very recently, in large doses interferon elicits a distinct effect in the treatment of patients with malignant neoplasms (Stringfell).

It should be noted that, aside from the main purpose, that of producing proteins of human origin that are in short supply, the advantage of this technology is that the end product costs considerably less than when it is isolated and purified from tissue or cell cultures. The main expenses are for scientific technical development to obtain highly active producer strains, while subsequent recovery thereof is one-tenth the cost using existing methods (T. I. Tikhonenko).

At the present time, the general trend in development of medical genetics is to increase the number of clinical disciplines that use methods of genetic analysis. Twenty years ago, medical genetics was represented mainly in

neurological practice; however, at the present time, there is not a single clinical discipline into which genetic ideas have not penetrated to some extent or other. The important tasks for clinical genetics is to study genetic heterogeneity of hereditary diseases, single out new nosological forms, work out diagnostic problems and identify the pathogenetic mechanisms upon which development of disease is based.

There has been quite substantial work done on new diagnostic methods and refinement of existing ones for hereditary diseases. In store for the near future is development of refined methods of biochemical, cytological and molecular genetic analysis of hereditary pathology. Computer technology will have to be used more extensively. All this will, no doubt, make it possible to single out many new hereditary forms of diseases. Special attention will have to be given to upgrading rapid diagnostic methods for mass scale examination of neonates and infants up to 6 months of age. Diagnostic tests for prenatal diagnostics must be expanded, as well as methods of diagnosing heterozygous carriers of mutant genes; the groups to be screened for X and Y sex chromatin will have to be expanded in order to detect patients with sex chromosome aberrations.

In the next few years, we can expect discovery of new patterns of correlation between genetic markers and environmental factors as risk factors that are instrumental in development of diseases with hereditary predisposition--so-called multifactorial diseases. Answers to these questions will help develop preventive measures against such diseases as atherosclerosis, cardiovascular diseases of other etiology, obesity, epilepsy, schizophrenia, etc.

Introduction of genetics in infectious disease practice is an important factor. It is known that development and outcome of bacterial and viral infections depend not only on pathogenicity of the pathogen, but so-called individual reactivity of the macroorganism. While these words previously implied something that was vague, at the present time reactivity refers primarily to concrete genetic factors that form the reaction of a given phenotype to infection. For this reason, studies related to investigation of genetic patterns of mechanisms of resistance of the organism to infectious agents are acquiring particular urgency in both the theoretical and practical respects. At the present time, quite a few experimental facts have been accumulated concerning the existence of an immunological component in development of such widespread diseases as atherosclerosis, malignant neoplasms, diabetes, collagenosis, etc. In this regard, it is a cardinal task for modern clinical genetics to determine the structural organization and mechanisms of immune system function. Determination of general patterns of structure and function of the immune system requires adequate models, and hereditary immunological diseases were expressly the foundation on which these problems are being solved so fruitfully at the present time.

Monogenic hereditary diseases with a known primary biochemical defect make it possible to trace the significance of a specific mutation to the function of immunocompetent cells and make a judgment as to the importance of disturbances referable to some metabolic cycle in the genesis of development of an immunodeficient state.

As an example, we can mention the studies of molecular mechanisms of formation of bactericidal systems of neutrophils (D. V. Stefani and Yu. Ye. Vel'tishchev). In the 100 years that have passed since I. I. Mechnikov discovered the phagocytic function of neutrophils, studies have not ceased in the direction of learning about the molecular bases of formation of bactericidal systems in these cells. Only in the course of investigations dealing with cells from patients with chronic granulomatosis, which is based on defective NADP•H_2 oxidase activity, the concrete molecular stage was disclosed of the mechanism of phagocytic activity of neutrophils.

It is common knowledge that therapy can be effective only if all aspects of pathogenesis of a disease and its etiology are known. This thesis was confirmed once more in the treatment of hereditary immunodeficient states. Injection of NADP•H_2 oxidase into granulocytes of patients with chronic granulomatosis made it possible to very effectively correct the level of completeness of phagocytosis in these cells (Tom and Jil). About 10 enzymatic hereditary defects have been demonstrated in granulocytes. These defects cause development of serious septic states (D. V. Stefani and Yu. Ye. Vel'tishchev).

Combined immunodeficient states with enzyme blocks in the cycle of purine metabolism constitute another important group of hereditary diseases (Seegmiller et al.). The very name of this state indicates that the pathological process involves the system of cellular and humoral immunity. Immunodeficient states with blocks referable to adenosine deaminase (Giblett et al.) and nucleoside phosphorylase (Osborne et al.) have been studied the most thoroughly in the molecular biochemical aspect. Development of septic states in the first months of life is the distinctive feature of these diseases. In 100% of the cases, without specific therapy infants die even with use of broad-spectrum antibiotics (Yu. Ye. Vel'tishchev and L. S. Balayeva). Identification of the primary biochemical defect provided a possibility for working out etiologically and pathogenetically validated therapy. This therapy elicited brilliant results. An erythrocyte mixture from healthy people is given to patients as a source of adenosine deaminase when this enzyme is blocked (May et al.), and this is sufficient for complete restoration of immunological reactivity. The foregoing convinces us of the need for differential diagnostics on the level of the primary defect and of use of specific [purposeful] therapy.

The next important direction of medical genetics, which is relevant to clinical disciplines, is the study of function of systems of DNA repair in groups of hereditary diseases that have a high risk for development of malignant neoplasms. Medical genetics has already made a substantial contribution in this respect in identifying hereditary defects in the system of DNA repair. One observes a drastic increase in tendency toward development of malignant neoplasms in the presence of a number of such diseases (V. M. Mikhel'son and N. V. Tomilin). Among them, we can mention Fanconi's anemia, Bloom's syndrome, ataxia-telangiectasis, xeroderma pigmentosum, etc. (V. M. Mikhel'son and N. V. Tomilin). Discovery of molecular defects in DNA repair with all of these hereditary forms, which are associated with a high incidence of malignant neoplasms, made it possible to draw with sufficient validation the conclusion of the cardinal importance of the state of the system of DNA repair to malignant transformation of cells (Setlow). The first indications have begun to appear about the

possibility of a defect in DNA repair in the case of atherosclerosis also (Hart and Setlow). It is imperative to assess the contribution of unstable genome function to pathogenesis of this widespread disease.

In addition to introduction of the fundamental advances of genetics to diagnostics, prevention and treatment of the above-mentioned widespread diseases, monogenic diseases also merit much attention. At the present time, the primary biochemical defect has been identified for more than 200 monogenic hereditary diseases (N. P. Bochkov). Knowledge of the defect makes it possible to undertake correction thereof on different levels of expression of genetic information of DNA, RNA and protein. In this respect too, gene engineering is ready, theoretically and experimentally, to formulate problems of "gene therapy" for cell cultures (T. I. Tikhonenko), whereas several experimental studies have demonstrated the possibility of transferring genetic information in experimental animals as well (Kline et al.). It is assumed that introduction of human genes with vectors, for example, of viruses and phages, into the patient's cells will elicit effective expression and correction of the defect. Subsequent "implantation" of this cured tissue to the patient should lead to a cure of his ailment. However, it should be noted that the fate of vectors (of viruses and phages) introduced into cells is not yet known, nor is the problem of quantitative effects of dosage of administered genes resolved; finally, the very transformation of eukaryote cells of foreign DNA is extremely inefficient (A. A. Bayev). All these questions must be answered in the near future.

Since the use of gene engineering approaches to patient treatment are still a matter of the distant future, we shall discuss other means of correcting genetic defects, some of which are already being used.

The principal methods of correcting hereditary defects, which have gained wide use in clinical practice, are based on the use of artificial diets to bypass the enzyme block. As an example, we can list numerous artificial diets consisting of synthetic amino acids or based on an assortment of natural products deficient in these amino acids. This form of therapy consists essentially of removing a concrete substrate, enzyme precursor that is deficient in a given disease from the diet or reducing the amount thereof in the diet (V. A. Tobolin et al.). In addition to diet therapy, stimulation of residual activity of the mutant enzyme is also used. This approach is effective in, for example, homocystinuria. Addition of large doses of vitamin B_6 to the diet, which is the coenzyme of homocystathionine synthetase, stimulated residual activity thereof to 5-6% of the control level, as compared to 0-1% in patients. In a number of cases, elimination of toxic products by means of complexons, for example, elimination of copper by means of penicillamine in the presence of hepatolenticular degeneration, is effective (Goldstein).

The above methods of correcting hereditary defects are not effective enough; they are used for a rather narrow range of diseases and, in a number of instances, they are not physiological (intolerance of synthetic diets).

Development of methods of replacement, etiologically validated enzymotherapy, is one of the promising directions of treatment. In the next few years, basically new methods will have to be developed for giving patients enzymes.

This is related to the fact that proteins injected in the blood stream are rapidly destroyed, poorly captured by cells and often elicit immunological reactions (Ye. F. Davidenkova et al., 1978a).

Development of new approaches consists of attempts to create microencapsulated enzyme products. At the present time, there are two main approaches to microencapsulation: insertion of macromolecules in natural capsules--erythrocytes and in artificial phospholipid vesicles, which were named liposomes (Sessa and Weissmann, 1968). The number of studies in these directions, starting with the first work on latency of enzymes in liposomes (Sessa and Weissmann, 1970), is growing constantly. In 1980, two monographs were published that dealt with liposomes (Tom and Jil) and there were three national symposiums. One of these symposiums convened in Moscow, in November 1980; a significant share of the papers delivered there consisted of research on development of liposome forms of insulin for treatment of diabetes by mouth and studies to correct hereditary defects (Ye. F. Davidenkova et al., 1981). At the present time, numerous technological schemes have been developed for producing microcapsules out of native and synthetic phospholipids. Capture of substance in liposomes has increased from 1-2% in the 1970's to 50-60%, and in model research there are also methods of 100% capture (O. A. Rozenberg et al.) of substances in microcapsules. Liposomes given via different routes, particularly intravenously, are well captured by organs that are rich in phagocytic cells (Ye. F. Davidenkova et al., 1978), primarily the liver, bone marrow and spleen (Tyrrell et al.). At the present time, the dual mechanism of liposome capture by cells has already been identified: either by means of phagocytosis of vesicles and their migration into lysosomes, or by fusion of liposome and plasma membranes (L. B. Margolis). The means of guided transport of liposomes have also been developed experimentally, by splicing to their membrane antibodies to target tissue (V. P. Torchilin et al.), due to local pH-dependent (Jatvin) or heat-dependent leakage of substances from vesicles (Weinstein et al.).

Effective penetration of liposomes into lysosomes of cells of the reticuloendothelial system (RES) is already being used in clinical practice for treatment of the adult form of Gaucher's disease. This disease is due to a block of glucocerebrosidase. Accumulation of substrate in lysosomes of RES cells leads to drastic enlargement of the liver and spleen, development of the syndrome of portal hypertension and death at the age of 25-30 years (L. G. Kalmykova). The response to treatment consists of drastic reduction in size of the liver, elimination of portal hypertension syndrome, etc. In addition to guided delivery of drugs to organs, liposomes also have the advantage that the material of the microcapsules, which is native, is not accumulated in the patient.

Intensive studies of this problem have been conducted in our laboratory since 1975. There has been experimental demonstration of the fate of liposomes injected intravenously (Ye. F. Davidenkova et al., 1978b) and possibility of correcting species-specific immunodeficiency of chicken leukocytes by administration of myeloperoxidase in liposomes (Ye. F. Davidenkova et al., 1981). The possibility of liposome capture by nonphagocytic cells was demonstrated in bacterial mutants. These studies, which we pursued together with the laboratory of S. Ye. Bresler, demonstrated the possibility not only of capture of the

liposome form of enzymes by bacterial cells, but correction of UV-endonuclease and DNA polymerase deficiency in the corresponding mutants. It must be stressed that the liposome form of administration of drugs opens up a new direction of pharmacology (Ryman et al.): it was shown experimentally that the efficacy of therapy of protozoan infections (Gregoriadis), chemotherapy of tumors (Rahman), antibiotic therapy (Gregoriadis), therapy of heavy metal poisoning (Rahman), etc., is drastically improved.

Microencapsulated drugs, which geneticists were among the first to use, turned out to be beneficial in many medical specialties. It can be assumed that there will be intensive development in the next few years of studies dealing with correction of hereditary defects on all levels of expression of genetic information.

We must stress the importance of further refinement of work referable to medical genetic consultation. This service must pursue comprehensive studies of the gene pool of the population, keep records on patients and their families, pursue exhaustive work in the area of forecasting the risk of development of hereditary forms of pathology among those examined and who sought consultation.

The tasks of this service also include speedy introduction and refinement of methods of biochemical and cytological diagnosis of hereditary diseases, dissemination of genetic information among physicians in other specialties and among the public.

The efforts of specialists in the field of genetics should be directed toward assuring the overtaking development of basic research. The tasks for clinicians consist of speedy adoption of these advances in diagnostic, preventive and therapeutic work referable to widespread diseases of man.

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HELIUM-NEON LASER USED TO TREAT ANGINA PECTORIS

Moscow KLINICHESKAYA MEDITSINA in Russian Vol 60, No 5, May 82 (manuscript received 8 Sep 81) pp 65-67

[Article by B. S. Agov, N. D. Devyatkov, A. Ye. Zhuk, N. S. Makeyeva, D. B. Tsykin and N. N. Shastin, Department of Faculty Therapy (headed by V. N. Latysh, doctor of medical sciences), Leningrad Sanitation and Hygiene Medical Institute]

[Text] A study is in progress in the Department of Faculty Therapy, Leningrad Sanitation and Hygiene Medical Institute, of the therapeutic effect of red light helium and neon laser (HNL) in patients with angina pectoris, which began in 1976. Experimental and clinical data on the beneficial effects of red HNL on metabolic processes in tissues, circulation and mitotic activity served as grounds for this study (U. Ya. Bogdanovich et al.; A. R. Rakhishev and B. Zh. Salimgereyeva; Ye. P. Chenskikh et al.). As shown by A. V. Borisov et al., exposure of the skin over the region of the rat heart was instrumental in dilating blood vessels, not only of the skin and subcutaneous cellular tissue, but the myocardium.

We submit here the results of our use of red light HNL for treatment of patients with angina pectoris over a 5-year period. In this time, the method of treatment was worked out, indications and contraindications for laser therapy were elaborated, studies were made of the effect of HNL red light on parameters of peripheral blood and the EKG: efforts were made to determine the mechanism of HNL effect on angina pectoris. The results obtained with the use of HNL over a 5-year period are consistent with the preliminary data we published previously (N. N. Shastin et al.).

HNL red light therapy was administered to 124 patients with ischemic heart disease and angina pectoris. There were 100 men and 24 women. The patients ranged in age from 33 to 76 years, most being over 50 years old (88 cases). Duration of illness was up to 5 years in 23 cases, 5-10 years in 55 and over 11 years in 46 patients. A total of 64 patients sustained myocardial infarction in the past, 45 of them once and 19 repeatedly. Daily attacks of tension angina pectoris were present in 66 patients, which were eliminated by keeping still or taking nitroglycerin tablets (first group of patients). A total of 50 patients suffered from tension and resting angina (second group); the attacks of angina were also corrected by nitroglycerin in these patients.

Unstable angina pectoris was present in eight patients; their pain was not always curbed by intake of nitroglycerin, and they were compelled to call for emergency care (third group). The first and second groups of patients took an average of 7 and 10 nitroglycerin tablets per day, respectively. In 74 cases, ischemic heart disease was associated with grade II essential hypertension.

HNL alone was used to treat 46 patients. The others received laser therapy in conjunction with anti-atherosclerosis and vasodilating agents, beta blocking agents, hypotensive agents and cardiac glycosides. We used a therapeutic laser unit, built on the basis of the industrially produced OKG-12-1 continuous action laser with a mixture of helium and neon, wavelength 0.63 μm, with energy density to the exposed surface of 0.2-0.4 mW/cm^2. A course of therapy consisted of 20 daily treatments with exposure of the sternal region, apex of the heart and collar region posteriorly, 10-11 cm in diameter, for 1 min to each region.

The results of treatment were assessed on the basis of changes in patients' well-being, quantity of nitroglycerin tablets taken, tolerance of exercise and EKG dynamics. Good results were obtained with laser therapy (discontinuation or lessening of angina attacks to less than one-half) in 26 patients (21%). A satisfactory effect was obtained in 81 patients (65%). In these cases, there was significant reduction of frequency and duration of anginal attacks, intake of nitroglycerin tablets was reduced and tolerance to exercise increased when performing the Master's test; nocturnal attacks of angina pectoris disappeared in a number of patients. There was no effect, or else it was insignificant in 17 patients (14%). Usually the patients felt better by the 3d-10th day of treatment, which warrants the belief that it is necessary to administer a course of HNL therapy consisting of at least 15-20 sessions.

As we demonstrated previously (N. N. Shastin et al.), the patient's age, duration of disease, history of myocardial infarction and concomitant diseases do not affect the results of laser therapy. The efficacy of treatment is clearly related to severity of angina pectoris. In milder cases (first group), treatment was effective in 89% of the patients and in more severe cases in 50%. HNL therapy was ineffective in all cases of unstable angina pectoris.

Brief worsening of condition (more frequent attacks of angina pectoris, increased number of nitroglycerin tablets used) occurred in 40 out 107 patients who presented a good response, between the 6th and 10th treatment. Thereafter, as therapy continued, they improved. We never had to stop laser therapy ahead of schedule because of intolerance of treatment. Worsening of well-being during treatment was observed reliably more often in patients who had a history of recurrent myocardial infarction. Transient worsening of well-being had also been noted in treating other diseases with lasers (V. M. Inyushin; A. N. Shabanov et al.). We failed to observe any side-effects from HNL red light therapy.

In a number of cases, improved well-being after the course of HNL therapy lasted for 6 months or longer (in 47 out of 107 patients). An effect lasting 1 to 3 months was noted in 60 patients; 33 patients who presented a prolonged effect initially underwent repeated courses of laser therapy with analogous

results. We did not repeat the course of laser therapy for patients who presented a brief response.

The effect of HNL on peripheral blood and EKG parameters is consistent with the changes in these parameters that we described previously (N. N. Shastin et al.). We failed to demonstrate appreciable change in pulse rate or arterial pressure, i.e., parameters characterizing cardiac function, after HNL therapy. In view of data in the literature concerning the effects of lasers on metabolic processes in tissues and dilatation of blood vessels in them, it can be assumed that the therapeutic response to this method of therapy is related to the effect on the microcirculatory system of the ischemic myocardium. However, this matter requires further investigation.

Conclusions

1. HNL light is an effective method of treating angina pectoris.

2. Optimum results were obtained with treatment of patients with tension angina pectoris.

3. Repeated courses of laser therapy can be recommended when a lasting effect was obtained from the first course.

4. Treatment with HNL light is not indicated for patients with unstable angina pectoris.

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CYTOGENETIC EVALUATION OF EFFECTS OF LASER RADIATION ON TOMATO CROP

Kishinev IZVESTIYA AKADEMII NAUK MOLDAVSKOY SSR: SERIYA BIOLOGICHESKIKH I KHIMICHESKIKH NAUK in Russian No 3, May-Jun 82 (manuscript received 3 Jul 81) pp 25-26

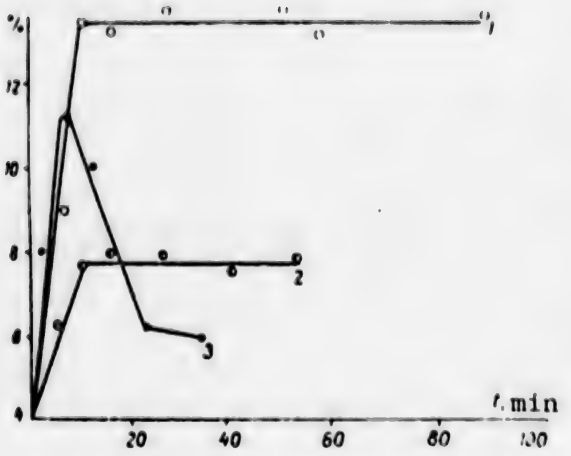
[Article by V. K. Burilkov and T. I. Saltanovich]

[Text] There are indications to the effect that most mutagens induce or inhibit crossing-over [1]. It is believed that expression of chromosomal aberrations and exchange of genetic material among homologous chromosomes are based on identical mechanisms. This hypothesis is based on the assumption that the same enzyme systems are involved in at least two processes, which are referable to both formation of mutations and crossing-over: chromosome break and reunion [3]. Consequently, the study of the effects of exogenous agents on the yield of chromosome aberrations would make it possible to assess the influence of these factors on activity of repair systems of the cell and, on this basis, to conduct a preliminary analysis of the feasibility of using a given agent as a recombinogen. (Previously, studies had been conducted to determine the mutagenic and physiological activity of laser radiation in corn, arabidopsis, tomatoes, etc. [5].)

Tomato rootlets 3-4 mm long served as the object of our investigation. We used LGI-21 (0.33 μm, R = 3 mW), LG-31 (0.44 μm, R = 20 mW) and LG-75 (0.63 μm, R = 15 mW) lasers as sources of radiation. After treatment, the roots were placed in an incubator at 25°C then fixed according to Carnoy after 10-12 h. Preparations were made by the propiono-resorcin blue method [4]. We counted chromosome aberrations in the anaphase.

When tomato roots are exposed to radiation at wavelengths in the visible range there was an increase in incidence of chromosome aberrations with increase in dose and subsequent saturation (see Figure, curves 1 and 2). The yield of aberrations as a function of dosage of ultraviolet laser radiation is a function with a maximum (see Figure, curve 3).

The assumption that laser radiation at the indicated wavelengths alters the activity of repair systems in the cell, thus causing expression of premutation changes in chromosomes that already exist in a limited quantity, could serve as one of the possible explanations for saturation of dose curves 1 and 2 [3]. However, in view of the fact that the spectra of effects of laser radiation in the given range of wavelengths and absorption of most cellular proteins



Yield of chromosome aberrations as a function of dosage of laser radiation. X-axis, time; y-axis, aberrations
1) λ = 0.44 μm, R = 20 mW
2) λ = 0.63 μm, R = 15 mW
3) λ = 0.33 μm, R = 3 mW

do not coincide, it can be assumed that lasers have an abscopal effect on the function of repair systems as a result of the following: change in membrane permeability and, consequently, impairment of ion balance, pH and energetic capabilities of the cell [2]; absorption of light by photoreceptive chromophore groups contained in proteins [2].

In view of data that distant ultraviolet light (>320 nm) damages cellular proteins, we cannot rule out the possibility of inactivation of repair enzymes and, consequently, increase in yield of chromosomal aberrations. The presence of a maximum for curve 3 is probably due to the fact that with significant doses of radiation some of the cells, which sustained many genetic injuries, are eliminated. For this reason, with increase in dosage there is a decrease in number of chromosomal aberrations observed in surviving cells.

Analysis of the spectrum of aberrations (retardation of one, two, three or more chromosomes, chromosome bridges) revealed that the relative number of chromosome bridges in the treated variants is 3-4 times greater than in the control. The presence of such aberrations is apparently related to occurrence of disturbances at the G_1 phase of mitosis [3].

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MEDICAL USES FOR LASERS

Moscow LENINSKOYE ZNAMYA in Russian 16 May 82 p 4

[Article by O. Semenova: "Lasers Cure"]

[Text] The specialists of the Moscow Oblast Scientific Research and Clinical Institute imeni M. F. Vladimirskiy were the first in our country to develop an effective method of treating a number of occupational diseases.

We all know that ultrasound has curative properties. It has become an irreplaceable physiotherapeutic agent, and it is now being actively introduced in the area of surgery. For example, ultrasonic "scalpels" have already appeared in many hospitals, and they are used for the most complicated operations. Physicians report that there is considerably better healing of incisions after using such instruments.

Nevertheless, a concern emerged: is not prolonged contact with ultrasonic equipment deleterious to health? This is far from an idle question. In recent times, ultrasound has begun to "work" with success in many sectors of the national economy--industry, science and transportation. For many people, it has become a familiar work tool. It is particularly popular as a highly effective method of nondestructive inspection of product quality. In heavy and power machinery building alone, up to 70% of the welded items are inspected by means of ultrasonic defectoscopy [flaw detection]. How do representatives of the vast occupation of defectoscopist-operator feel at the end of a work day?

The staff of a Problem Commission at MONIKI [Moscow Oblast Scientific Research and Clinical Institute imeni M. F. Vladimirskiy], which was founded by order of the USSR State Committee for Science and Technology, undertook an analysis of this situation. In general, ultrasound is harmless in low doses and even, as we know, beneficial. However, the conclusion made in the laboratory was disquieting: the very same ultrasound that treats (and the radiation dose in industrial installations does not exceed therapeutic levels) could cause some diseases if there is prolonged and periodically repeated contact with it. Expressly it is to blame for development of pain in operators' hands, heightened sensitivity of the skin to cold and vegetovascular syndromes.

The fact of the matter is that transformers installed in the probe of a unit are used by inspectors ["defectoscopists"] for generation and reception of

ultrasonic waves. The operator uses them to inspect products, thereby exciting ultrasonic waves at different points of surfaces, but part of the wave energy hits the worker's hands. This is what causes pathological changes in tissues, vessels and nerve endings.

Scientists began to search for a means of protecting people against ailments. They had to spend about 10 years to find the right solution.

Now this search is behind us. I am talking with a patient in one of the wards in the occupational pathology clinic of MONIKI, who is being discharged in a few days. Anna Ivanovna K. has a considerable work tenure in the ultrasonic defectoscopy department. She was admitted to the clinic complaining of sharp pain in the hands, which prevented her from lifting even a light shopping bag.

"Now I feel quite decent," says Anna Ivanovna. "I think that I will be able to continue working in my special field."

"We 'armed ourselves' with lasers to control occupational diseases," explained later A. B. Chemnyy, candidate of medical sciences, head of the problem laboratory for the study of physical condition of defectoscope operators. "They have yielded excellent results. Over 90% of the patients who underwent two courses of laser radiation return to their former jobs."

Physicians have long since noticed that even 2-3-min exposure to laser light at a specific frequency has a biostimulating effect. The first rather approximate experiments with the use of "lasers against ultrasound," which were conducted at MONIKI, have yielded positive results.

The creative collaboration of clinicians with electronics designers has largely facilitated solving the problem. Taking into consideration the reserachers' wishes, they developed a precise and convenient physiotherapeutic unit. Last year, series production thereof was set up. Soon it will appear in hospitals, polyclinics and preventoriums.

However, laser therapy is only part of the large job done by the staff of the laboratory. They have done very much to prevent diseases. The first step that they took in this direction was to "bring closer" treatment to patients, having organized an experimental prevention center at the Podol'sk Machine-Building Plant imeni S. Ordzhonikidze.

The Moscow physicians helped their colleagues in Podol'sk to outfit a special preventive office and trained specialists. In addition to the laser unit, the health center was equipped with a thermograph ["thermovision unit"], which is an instrument that permits observing the condition of soft tissues. For the symptoms of many diseases caused by ultrasound are so unnoticeable at the early stage that it is simply impossible to make the correct diagnosis without the thermograph.

... It is the dinner break at the Plant imeni S. Ordzhonikidze. There are always many visitors at the health center at this time. And this is understandable. A small line has already formed at the door of the prophylactic office. However, no one is anxious; the procedure takes very little time. People talk unhurriedly and joke.

"Who is last for lasers?"

Here, people are treated as if they were at home.

"Operation of this prophylactic office yields a significant economic effect to the enterprise," stated N. I. Nikitina, industrial health physician. "Prevention is easier than treatment, physicians like to say. And we are trying to prevent development of diseases."

The knowhow of prophylactic offices, like the one in Podol'sk, is beginning to spread over this oblast. For example, a similar center is being organized at the Stupino Metallurgic Combine.

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HELIUM-NEON LASERS IN TREATMENT OF PATIENTS WITH ODONTOGENIC INFLAMMATORY DISEASES

Alma-Ata ZDRAVOOKHRANENIYE KAZAKHSTANA in Russian No 6, Jun 82 pp 42-44

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[Text] The main objective of treatment of acute suppurative and inflammatory diseases is to limit the spread of the infectious process. This is achieved by reducing virulence of the infectious factor, increasing immunological reactivity of the organism and immunological properties of tissues in the region of the jaws and face. Prompt elimination of sites of odontogenic infection, lancing of abscesses and phlegmons, ordering antibacterial, pathogenetic agents and physiotherapy are recommended to correct disturbances of blood and lymph circulation in the jaws and soft tissues, which occurs with odontogenic inflammatory diseases. In this regard, the clinical use of laser therapy, as one of the promising methods of physiotherapy, is of certain interest. There have been good results with the use of helium-neon lasers (HNL) in the treatment of a number of stomatological diseases in both adults and children: pulpitis, periodontosis, stomatitis (D. L. Korytnyy; M. T. Aleksandrov et al.; L. A. Lutsyk et al.). However, we failed to encounter in the literature any works dealing with the use of HNL for treatment of patients with odontogenic inflammatory diseases.

Clinical use thereof was preceded by experimental work. Studies were pursued of the effects of laser therapy on aseptic staphylococcal infection in the region of the rabbit's mandible, and determination was made of the possibility of using a combination of radiation with surgical intervention in the site of inflammation (experiments were conducted on 340 animals weighing 1800-2600 g). A control group was formed for each series. Aseptic inflammation was produced by hypodermic injection of 0.1 mℓ 0.5% crotonic acid. To produce focal staphylococcal infection, a 24-h culture of a stock strain of pathogenic staphylococcus was used in a dosage of 2 billion microbial bodies per milliliter. The site of inflammation was exposed once to an LG-75 laser with output of 20-22 mW at a wavelength of 0.63 μm for 3 min. Two modes were used: early exposure (30 min after giving stimulating agents) and on the 3d day of development of

inflammation. We assessed the efficacy of laser therapy on the basis of hematological, biochemical, immunological blood tests and morphological examination of soft tissues in the region of the inflammatory focus. The results of these studies were submitted to statistical processing.

As compared to results obtained in control groups of experimental animals, there was considerably less development of inflammatory infiltrate under the influence of early single exposure to HNL. We demonstrated moderate leukocytosis, lymphocytosis, a fibrinolytic effect and activation of nonspecific defense factors, which is what prevented development of more profound changes in blood serum protein fractions.

Microscopically, there was a tendency toward circumscription of the inflammatory process at the early stages (12 and 24 h). While a diffuse process was demonstrable in nonirradiated animals at 48-72 h, involving the subcutaneous tissue and spreading into the dermis, with use of HNL the process was circumscribed; it was not associated with infiltration of dermis and presented separation of young granulation tissue or a demarcation zone of severely hyperemic vessels. On the 5th-7th day, in the case of aseptic inflammation, the subcutaneous cellular tissue showed bands with a marked three-layer capsule (pyogenous membrane, juvenile and maturing granulation tissue) and residual signs of productive inflammation in the dermis. In the case of staphylococcal inflammation, there was a tendency toward intensification of proliferation in the dermis and subcutaneous tissue. In particular, abscesses were circumscribed, with well-formed maturing granulation tissue; there were marked trabeculae in subcutaneous cellular tissue and lower regions of the dermis. Cicatricial tissue consisting of coarse fiber connective tissue was found in the subcutaneous tissue on the 10th-15th day. Studies pursued on the 30th and 45th days revealed that there was better healing of the inflammation site under the effect of HNL and it consisted of mature connective tissue. The cicatrices were smaller in irradiated animals, whereas in nonirradiated ones they consisted of coarse hyalinized fibers.

Exposure of inflammation sites to HNL on the 3d day of disease was instrumental in drastic limitation of the suppurative process; it caused active demarcation hyperemia of vessels and formation of young granulation tissue. On subsequent days, there was formation of circumscribed inflammatory infiltrates; they were resorbed faster and cavities replaced with cicatrices of connective tissue.

Intensification of repair regeneration was demonstrable from the first day after lancing an abscess and irradiating the wound. In particular, there was intensive proliferation of strata spinosum and basale of the epidermis, hyperplasia of hair bulbs, proliferation of histiocytes, plasmocytes and fibrocytes. The suppurative focus was circumscribed by a region of plethoric vessels and a two-layer capsule consisting of fibrous connective tissue, many fibrocytes, macrophages and leukocytes. The postoperative wound healed with complete epithelialization and formation of delicate cicatrices of connective tissue.

Thus, early treatment of an inflammatory site with HNL had a favorable effect on the course of disease. Irradiation of this site at the height of the disease accelerated formation of an abscess, limited its size and was instrumental

in faster resorption thereof. The combination of surgical intervention and laser therapy accelerates processes of repair regeneration of a suppurative wound.

The positive results of experimental studies served as grounds to use laser therapy for treatment of 35 patients with odontogenic inflammatory disease. The patients ranged in age from 7 to 76 years. There were 4 children and 30 adults. One female patient was 76 years old. There were 18 males and 17 females. Maxillary periostitis was diagnosed on the basis of clinical and roentgenological examination in 18 cases, abscesses of perimaxillary soft tissues in 14 and osteomyelitis of the jaws in 3. The sources of odontogenic infection were found to be an inflamed mucous membrane in the retromolar region with difficult cutting through of the lower third molars (16 cases), periodontitis of permanent (14) and deciduous (3) teeth at a stage of exacerbation, developed state of periodontosis (2 cases).

Treatment was administered on an out-patient basis. Surgical care was rendered as emergency therapy: extraction of involved tooth and lancing of suppurative foci (28 cases), lancing of suppurative foci followed by conservative treatment of involved tooth (5 cases). Two patients with a protracted inflammatory process were referred for additional treatment after therapy in the hospital. Antibacterial and pathogenetic therapy was ordered when indicated. Laser therapy was administered under dosimeter monitoring, in accordance with the methodological instructions worked out by the Main Administration for Therapeutic and Preventive Care, USSR Ministry of Health (A. A. Prokhonchukov et al.). We used the LG-75 laser with output power of 16-15 mW. Laser radiation was delivered to the inflammation site by means of a mirror-lens light guide. Exposure to HNL per field constituted 1-2 min, depending on the patient's age and type of odontogenic inflammatory process. In the presence of a diffuse inflammatory process in the bottom of the buccal cavity, the site of inflammation was irradiated in two points: on the side of the buccal cavity and through the integument. Total exposure time constituted 2-3 min per session; a course of therapy consisted of 3-5 daily sessions.

A positive effect was obtained with laser therapy in all patients. After 1-2 sessions, there was reduction or disappearance of pain, normalization of body temperature and improved well-being. At the site of inflammation, there was appreciable reduction of edema and infiltrate of soft tissues, which were completely eliminated after completing a course of therapy. Pus was found in the incisions during surgery on 29 out of 33 patients. After 1-2 sessions of laser therapy, suppurative discharge stopped in 27 cases and in only two patients continued for 3-4 days. Healing of the alveolus of the extracted tooth occurred at the usual time. We failed to observe lysis of blood clot or development of alveolitis. A marked clinical response was observed in patients with inflammatory contracture of masseter muscles. Here is a case history: Patient S., 37 years of age, was treated in the hospital from 5 to 14 December 1981 for phlegmons in the submasseter ["subchewing"] space to the right of $8^{\overline{1}}$.* The phlegmon was lanced and the involved tooth was extracted. The patient received antibacterial symptomatic therapy and a course of UHF therapy. His condition improved. Upon discharge, there was a marked firm infiltrate in the parotid-masseter and retromolar regions. He could open his mouth to 1 cm.

*Lower right wisdom tooth.

Laser therapy to two points was ordered: to the parotid-masseter region for 2 min at a time and retromolar region for 1 min at a time. The course of therapy consisted of five sessions. After treatment, the mouth opened freely. But a small infiltrate remained. Disability lasted a total of 20 days.

Examination of clinical blood tests failed to demonstrate an adverse effect from laser therapy. Parameters were in the range of physiological fluctuations.

Thus, our clinical and experimental studies demonstrated that laser radiation has a marked anti-inflammation and stimulating effect, which permits recommending this type of therapy for patients with odontogenic inflammatory diseases.

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ACCIDENTAL LASER INJURY TO FUNDUS OF BOTH EYES

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[Article by V. M. Gayday, candidate of medical sciences, Lt Col Med Serv, and V. I. Filippenko, candidate of medical sciences, Col Med Serv]

[Text] The area of application of lasers in different sectors of technology, science and medicine has broadened considerably in recent years; for this reason, there has also been an increase in the range of specialists related to production, adjustment and operation thereof. As it was learned, the biological effect of laser radiation on man is referable primarily to the organ of vision.

Analysis of data in the literature shows that if there is infraction of safety rules when working with lasers, they are accidentally turned on and even while monitoring the operation of lasers without special protective goggles, one can sustain eye damage varying in severity, with partially reversible or irreversible loss of visual functions. The effect of laser beams on a biological system is based on a thermal burn, coagulation of protein structures and even mechanical trauma.

The degree of change in tunics of the eye exposed to lasers depends primarily on the dosage of laser radiation. Different authors cite quantitatively different threshold values for intensity of radiation from pulsed lasers. R. A. Besyadovskiy (1965) observes that one can consider 0.01 calories/cm^2 as the maximum energy of radiation that does not elicit damage to the tunics of the eye. According to V. P. Zhokhov (1971), the threshold energy density on the retina constitutes 0.7 J/cm^2 for a ruby laser in the free generation mode and 2.6 J/cm^2 for a neodymium laser. The nature of damage to tissues may depend not only on the intensity of radiation, but exposure to a pulse, degree of pigmentation of the fundus, width of pupil, transparency of medium in which the beam is propagated, distance, etc.

When the laser operates in the free generation mode, the radiation pulse has an exposure on the order of $1 \cdot 10^{-3}$ s. With supraliminal energy densities, thermal damage to the retina and choroid is manifested not only by coagulation of protein, but eruption of fluid in surrounding tissues. In addition, there is mechanical trauma to the tunics of the eye. Thus, not only does the fundus sustain a burn, but there are ruptures in the retina, occasionally with hemorrhages in the region of injury.

One should consider it more dangerous to sustain injury by a laser operating in the Q-switched mode. In this case, the radiation pulse is considerably shorter and exposure is measurable in values on the order of $1 \cdot 10^{-9}$ s. Here, heat no longer extends to surrounding tissues, and there is instantaneous heating of biological structures at the site of injury to very high temperatures, which leads to microruptures [explosions?] that eject particles of torn retinal tissue in different directions, including the vitreous body (B. L. Polyak, 1972).

To date, there is a sufficient number of observations in the literature, on the basis of which it can be concluded that not only direct, but reflected and even scattered laser beams have a deleterious effect on the eye (A. A. Komarova et al., 1978).

V. P. Zhokhov et al. (1971) observed two cases of injury to the fundus of the eye by laser radiation in physicists. In both cases, there was monocular eye damage, with relatively good functional outcome and anatomical sequelae in the fundus, in the form of atrophic foci. There are also reports by other authors (L. A. Linnik et al., 1978; G. I. Osipov, M. M. Pyatin, 1978) who mention severe destructive damage to the macular region due to laser radiation, with irreversible loss of visual functions.

The case we observed of severe damage to both eyes from a direct laser beam is of practical importance.

Patient P., 22 years of age, while performing geodesic work, adjusted the optical system to the marker of a quantum range finder in a routine check of orientation of a theodolite at a distance of 350 m and got close to the eyepieces. At this moment, his associate who was working with a laser range finder, "fired a shot" over the theodolite's optical system, thus infracting safety practices. P. experienced a sudden severe impact, a bright pink flash before his eyes, diminished vision in both eyes and a sharp pain in the eyes (more so in the right one). Large black spots appeared before his eyes and, in addition, a veil-like film was formed in the right eye, which prevented him from seeing objects. The film disappeared in about 3-4 days, and a permanent spot appeared before the right eye.

On the second day after this occurrence, visual acuity was 0.1 in the right eye and 0.6 in the left, and could not be corrected (initial visual acuity was 0.1 for both eyes, with emmetropia). Ophthalmoscopically, there was a gray focus in the region of the right macula lutea, without circumscribed boundaries and with fine hemorrhages; a parafoveal focus of a gray macula with diffuse margins was visible in the left eye.

Osmotherapy was administered to the patient using intravenous injections of 40% glucose solution with 5% ascorbic acid and 10% calcium chloride solution in the usual dosage, as well as electrophoresis to both eyes successively with hyaluronidase [lidase] and aloe. To improve metabolic processes in the retina, ATP was given intramuscularly at the rate of 2 ml (20 injections), vitamins A and C were given by mouth, and for biostimulation injections of aloe (45) and apilacum tablets.

After 3 months, visual acuty of the right eye was 0.1, eccentrically, not corrected, with emmetropia. Visual acuity of the left eye was 1.0, with emmetropia. Perimetric and campimetric examination revealed an absolute central scotoma of the right eye, which was almost circular, 8-10° in size. There was no impairment of color discrimination in both eyes, according to monocular testing with polychromatic tables. Dark adaptation (3-min adaptometry) was 25-30 s for the right eye and 15-20 s for the left.

Right fundus: normal macular reflex absent; a perforated defect in the region of the macula lutea, including the central fovea of the retina, going almost to the sclera, strictly circular in shape, about 1/2 RD in size, with connective tissue proliferation around the perimeter and in the center an inclusion of a clump of pigment around a ray-shaped cicatrix.

Left fundus: site of macular atrophy in the parafoveal region up to 1/4 RD in size with pigment inclusions. Refracting media--cornea, lens and vitreous body--are clear.

The patient complained of drastic reduction of vision in the right eye, presence of a constant spot before the eye, in the center, distorted shape and reduced size of objects and yellowish tone thereof. Before the left eye, he experienced a paracentric dark spot with several lines.

Thus, in the case we have described, there was serious damage to the macular region of both eyes by radiation from a laser range finder. The distinction of this case, as compared to those described in the literature, is the binocular involvement of the eyes in damage from an optical instrument at a great distance. The severity of macular burns was attributable, in our opinion, not only to the eyes' natural capacity to focus a beam of light in the region of the central fovea of the retina, but the optical system of the transit. Even actively administered therapy was ineffective, and this is entirely consistent with data in the literature (L. A. Linnik et al., 1978).

One should consider development of means of individual and group protection, unfailing adherence to safety rules by all specialists working with lasers to be the main direction to follow for the prevention of this type of ophthalmological traumatism. Well-planned and constant health education work is required of medical workers.

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ANATOMICAL STRUCTURE AND TOPOGRAPHY OF PORPOISE ESOPHAGUS

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[Article by V. Ya. Lukhanin, Institute of Zoology imeni I. I. Shmal'gauzen, USSR Academy of Sciences]

[Text] There is information in the literature concerning the structure of the esophagus of some cetaceans (Yablokov, 1958; Kleynenberg, Yablokov, Bel'kovich, Tarasevich, 1964; Harrison, Johnson, Young 1970; Yablokov, Bel'kovich, Borisov, 1972; Green, 1972; Simpson, Gardner, 1972). But it consists mostly of scattered data obtained for different species of cetaceans. We have tried to conduct a fuller investigation of the esophagus of a specific cetacean species, the porpoise (Tursiops truncatus ponticus B.).

In this report, we submit data on macromorphology and, in part, micromorphology, as well as topography of the porpoise's esophagus. The material was taken from 12 animals; we used carcass sections according to N. I. Pirogov and prepared them according to V. P. Vorob'yev; histological preparations were stained with hematoxylin-eosin.

The porpoise's esophagus, which is a segment of the digestive track that extends in the form of tube between the pharynx and stomach, starts at the level of the atlantis-occipital articulation and ends, moving into the stomach, on the level of the first lumbar vertebrae.

The first or cervical part of the esophagus (pars cervicalis oesophagi) is rather short and situated in the region of the neck. The middle or thoracic part of the esophagus (pars thoracalis oesophagi) is the longest, extending from the site of entrance of the esophagus through the anterior thoracic orifice into the thoracic cavity to the point where it penetrates through the esophageal orifice of the diaphragm into the abdominal cavity. The last, or abdominal part of the esophagus (pars abdominalis oesophagi) is the shortest, and it is situated in the abdominal cavity from the site of penetration through the diaphragm to the stomach. The first and last segments of the esophagus are compressed by adjacent organs in a dorsoventral direction and the central part in a lateral direction.

Over virtually its entire length, the esophagus is situated along the midline ventrad to the spinal column and only its last part deviates somewhat to the left and even more ventrally.

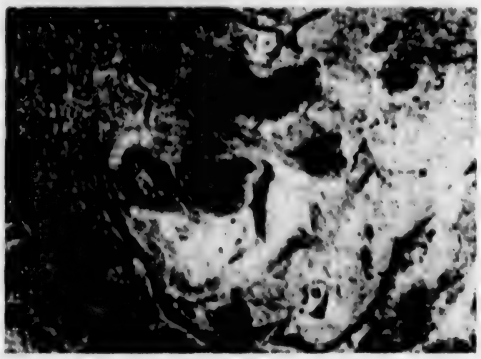

Figure 1. Location of esophagus in relation to adjacent organs (frontal section, posterior view, photo of preparation)

1) esophagus
2) trachea
3) aorta
4) left lung
5) right lung

Dorsally, the esophagus is adjacent to the spinal column, there being a layer of friable cellular tissue between them in which is located the prevertebral part of the vascular rete mirabile. In addition to the spine, the thoracic aorta is also on the boundary of the middle and posterior segments. Almost at the diaphragm proper, the esophagus deviates completely away from the spine (Figure 2), to the left and ventrally. There it is dorsally adjacent to the aorta, prediaphragmetic central lymph node and left lung.

Ventrally, the first segment of the esophagus is adjacent to the dorsal wall of the trachea (Figure 1) and, after the tracheal bifurcation, to the bronchi. From the site of tracheal bifurcation, the dorsoventral flattened part of the esophagus changes into the lateral part. The lower part of the esophagus descends even more ventrally in the space between bronchi.

Caudad to the tracheal bifurcation, the esophagus is ventrally adjacent to pulmonary vessels on the level of the 3d-4th thoracic vertebrae. Then, on the level of the 4th-7th thoracic vertebrae, it is adjacent to the pericardium covering the dorsal part of the left atrium and ventricle, and to the posterior vena cava.

Even more caudally, the esophagus is surrounded on the bottom and the sides by the prediaphragmatic lymph node on the level of the 10th-11th thoracic vertebrae (Figure 2), and through it bordering on the diaphragm and liver. Over virtually its entire length, the thoracic segment of the esophagus is also partially adjacent to the lungs at the bottom.

The sides of the first part of the esophagus are adjacent to cervical muscles, neurovascular bundles, as part of which the left and right common carotid arteries (a. a. carotis communis dextra et sinistra), left and right vagi (n. n. vagus dexter et sinister) pass. Somewhat caudally to the right of the esophagus, there is an adjacent preaortic bronchus coming off the trachea on

the level of the last cervical and first thoracic vertebrae and then, on the left, the aortic arch that goes around the trachea and esophagus. Even more caudally, the esophagus borders on the thoracic aorta on the left. The latter, going under the trachea and esophagus, extends obliquely in a dorsocaudal direction to the left of the esophagus (Figure 1). In addition to these organs, the lungs are adjacent to the esophagus over almost its entire length, and esophageal indentations are evident in the lungs in the adjoining segments.

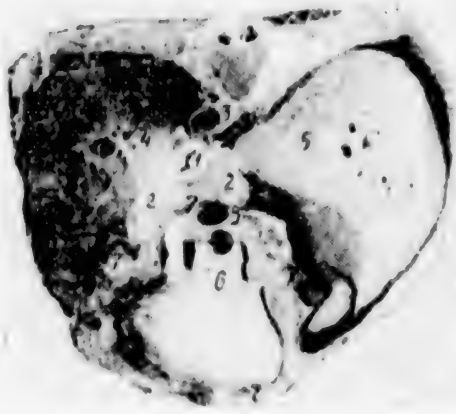

Figure 2.
Frontal section of esophagus (posterior view, photo of preparation)
1) esophagus
2) prediaphragmatic medial lymph node
3) aorta
4) left lung
5) right lung
6) liver
7) diaphragm
8) posterior vena cava

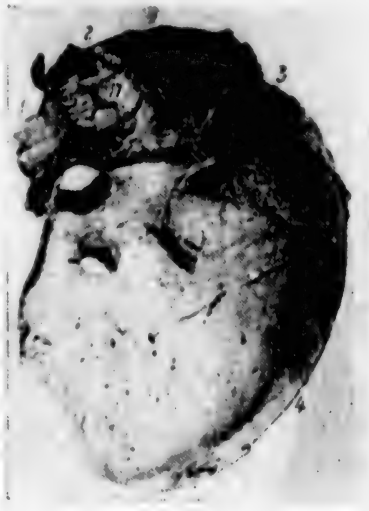

Figure 3.
Esophagus on boundary between thoracic and abdominal cavity (frontal section, anterior view, photo of preparation)

1) lymph node
2) esophagus
3) diaphragm
4) liver

In the abdominal cavity, the esophagus, which narrows down appreciably, penetrates through the esophageal orifice of the diaphragm. Here, for some distance the esophagus is covered on all sides by the diaphragm (Figure 3). Then, caudally it gradually is released from the diaphragm, first on the left and then the central side (Figure 5). Dorsally and on the right, the diaphragm covers the esophagus all the way; moreover, it passes to the corresponding surface of the vestibulum [pyloric?] of the stomach (Figures 4 and 5). Dorsally, this part of the esophagus borders through the diaphragm with the posterior segment of the left lung and thoracic aorta. The latter leaves a longitudinal depression on the adjoining segment of the esophagus.

Ventrally, the esophagus adjoins the liver, first being separated from it by the diaphragm (Figure 3). Caudally, after disappearance of the diaphragmatic leaflet at the bottom, the esophagus adheres directly to the liver, forming a corresponding indentation in it (impressio oesophagea).

Figure 4.
Reciprocal positions of abdominal part of esophagus and second segment of stomach (posterior view, frontal section, drawing of preparation)
1) second segment of stomach [reticulum]
2) diaphragm
3) esophagus
4) posterior vena cava
5) liver

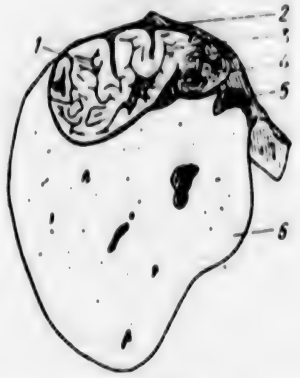

Figure 5.
Correlation between esophagus, anterior diverticulum of gastric vestibulum and second segment of stomach on the level of the vestibulofundal orifice (posterior view, frontal section, drawing of preparation
1) second segment of stomach
2) diaphragm
3) anterior diverticulum of vestibulum
4) esophagus
5) posterior vena cava
6) liver

The anterior part of the second segment of the stomach adjoins on the left the esophagus, which penetrates through the diaphragm (Figure 4). Then the left anterior diverticulum of the gastric vestibulum is wedged between them (Figure 5).

A distinct mucous membrane (tunica mucosa) is well-visible on cross sections of the esophagus (Figures 1-3). There are 5-7 tall folds of mucosa oriented longitudinally that can be clearly discerned. As they come close to one another, these folds fill the lumen of the esophagus. The mucosal folds are well-marked because of the submucal layer that is involved in their formation. The mucosa is covered with stratified squamous epithelium with signs of hornification in the superficial layers. The latter imparts a whitish cast to the esophageal mucosa. The papilla of the mucosa proper, which consists of fine fibrillar tissue containing reticular elements, pass deep into the epithelium that covers it.

In the mucosa propria, one encounters small lymphoid collections. Then there are longitudinally oriented fascicles of smooth-muscle fibers of the muscular layer of the mucosa.

The submucosa (tunica submucosa) is developed rather well; it consists of friable connective tissue with a few elastic fibers. Mucous glands, vessels and nerves are situated in it.

The esophagus has a rather well-developed muscular tunic (tunica muscularis) with internal circular and external longitudinal layers. In the cranial part of the esophagus, it is a continuation of the striate muscles of the pharynx. We

observed gradual replacement of some striate muscle fibers with smooth ones in the central part of the esophagus. The smooth muscle fibers prevail in the caudal part of the esophagus.

In the external fibrous tunic (tunica adventitia), there is prevalence of fibrous tissue, with insignificant amount of elastic fibers.

The information on topography of the esophagus is original. In conclusion, it must be noted that the esophagus is referable to organs that were less subject to alterations that occurred in cetaceans because of their secondarily marine mode of life.

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BIODEMOGRAPHIC ASPECTS OF SCIENTIFIC AND TECHNOLOGICAL PROGRESS

Moscow VESTNIK AKADEMII MEDITSINSKIKH NAUK SSSR in Russian No 4, Apr 82 (manuscript received 30 Oct 81) pp 74-78

[Article by A. T. Shatalov (Moscow)]

[Text] At every new stage of advancement of society, science and technology impose their specific imprint on some aspects of its development. The present stage is notable, in particular, for the ever increasing influence of scientific and technological progress on the structure and dynamics of biodemographic processes. This is reflected in acceleration of development of the young and retardation of aging of the elderly generations, decline of birth rate, etc.

Of course, the content, direction and pace of such changes in different countries and economic-geographic regions depend not only on changes in the integral biodemographic structure of mankind and development of science and technology, but on socioeconomic, political transformations of society and condition of the environment. It is also important to stress that the socioeconomic factor was and still is generally the leading one in the complex set of effects of diverse factors on the structure of biodemographic processes. However, this does not preclude the need for special analysis of determination of new trends in development of biodemographic processes by scientific and technological circumstances as well. Consideration of the results of such analysis could play a substantial role in practical implementation of comprehensive and harmonious development of society, its natural prerequisites and conditions. Successful performance of this task will open up new prospects for further scientific-technological and general social progress, as well as progress in correlations of society and its natural biodemographic basis.

Let us examine briefly those of the above trends that are related to the ever increasing influence of current scientific and technological progress on various structural elements of biodemographic processes. Such trends include acceleration of physical development of children and adolescents, retardation of aging of the elderly and aged, postponement of actual old age to a later time.

The acceleration phenomenon consists primarily of a faster rate of physical maturation of the rising generations. Numerous studies of scientists (V. Bunak;

V. Solov'yeva; R. Noybert; G. Grimm; K. Vinter and G. Shtrass, and others) show that modern children and adolescents develop faster physically than their peers who lived 70-80 years ago, and they present higher anthropometric and physical parameters (height, chest circumference, weight, etc.). They become physically mature 2-3 years earlier. There is a tendency toward earlier puberty, particularly in girls, as a result of which there is intensification of sexual dimorphism. There is reason to believe that all this is a distinctive biological reaction of adaptation to a number of social and socially determined processes (improved material welfare of modern generations in general and young people in particular, considerable increase in volume of diverse information about relations between the sexes and availability thereof thanks to television, movies, radio, the press, etc., advances in medicine and national health services in the area of safeguarding the health of children and adolescents).

At the same time, acceleration of physical development of current generations of children and adolescents is not related in every respect to the pace of their social and spiritual maturation. Several researchers (G. Tsaregorodtsev; T. Karsayevskaya and others) mention the presence of a discrepancy between times of physiological and social-spiritual maturation; the latter often occurs later than the former, which sometimes leads to undesirable consequences (including social ones). Hence the extremely complex problem of eradicating such a discrepancy, and it is even more difficult to solve it because of extension of the period of education and acquisition of diverse knowledge, skills, social values, the quantity of which is constantly growing.

In making an analysis of the influence of scientific and technological factors on acceleration of young generations of people of different age groups, one must take into consideration the distinctions of development of the adult human body considered both as a whole and on the level of different systems--cardiovascular, nervous, musculoskeletal, etc. This is particularly important to pedagogics, psychology, medicine and age-related physiology, which have a direct bearing on the problem of physical development of rising generations. The fact of the matter is that not all of the systems and functions in the child, adolescent and young person are formed and developed synchronously at an accelerated pace. The effects of exogenous factors can intensify this asynchronism to undesirable and even dangerous extents. Thus, one sometimes observes some retardation (deceleration) in development of the cardiovascular system, as compared to other systems of the body, in the presence of kinesthetic deficiency related to the inactive life-style of some young students. When there are excessive loads on the nervous system due to the rapid pace of life in modern cities, changes in content of labor (in particular, reduction of share of physical labor and increase in share of mental labor) and increase in general flow of information that is not sufficiently organized, stress situations are not uncommon, and they cannot fail to have an adverse effect on nervous system function which, in turn, leads to greater asynchronization of accelerated development of the other systems. In antagonistic [social] systems, stress is aggravated by exacerbation of class contradictions, fear of unemployment, threat of economic, ecological, financial crises, etc.

In view of the foregoing, it is now time to find a cardinal solution to the problem of harmonizing scientific and technological process with sociohygienic progress, which requires utmost consideration of compensatory capabilities of

the human body and its system in the changing environment, control of chronic diseases and traumatism. Satisfaction of this need is of substantial importance to the development of the next generations, which inherit from preceding ones not only material and spiritual values, but certain biological elements (constitutional, capacity for longevity, predisposition for certain diseases, etc.).

Accelerated physical development of the young (like retardation of biological, actual time of occurrence of old age, which we shall return to later) creates favorable conditions for prolonging the active period of life of current generations of people, which is characterized by greater harmony of their physical, psychological and social development, as compared to other age periods. The level of such harmony is not only indirectly, but directly related to socio-economic, scientific-technological and social-ecological-hygienic conditions.

There is an obvious need to expand and intensify research directed toward further prolonging the active period of people's life, which requires that an increasing number of specialists in the natural and engineering sciences, as well as humanities, work on relevant problems. This is particularly important to the solution of the problem of manpower resources, which has become acute in the USSR in recent times, as well as more effective and differentiated utilization of man's innate inclinations.

The next trend is retardation of aging (slowing of the aging process during the period of old age). Physical development and especially spiritual development of modern generations of older age groups is characterized by a slowing of involutionary and degenerative processes. In addition to lowering the mortality rate, this slowing has resulted in prolonging the life of the elderly and aged. They retain the capacity to work (entirely or partially) for a considerably longer time than generations of the 19th and earlier centuries. The presence of this new trend (along with the increase in life expectancy) has resulted in significant "aging of the population" of many economically developed countries in the second half of our century, as manifested by absolute and relative growth in share of elderly and aged individuals in the age-related structure of the population as a whole and workers in particular. The "old" countries in the demographic sense of the word include those where the share of individuals over 60 years of age equals or even exceeds 12%. The trend of demographic aging was first discovered about 100 years ago in France. In our times, the share of elderly people is close to 15% in many countries of Europe and the USSR.

According to the estimate of E. L. Rosset (Polish People's Republic), there were 280 million people 60 or more years of age over the entire world, in the 1970's, i.e., 7.6%. Forecasts predict a further increase in this age group. According to WHO data, in the year 2000 the number of individuals 60 or more years of age will be 585 million, i.e., there will be an increase from 8 to 9% in share of old people over the entire world.

In describing our era, in which the modern world lives, among its main changes there is indication of technological transformations (era of the atom and space), social (era of communism) and demographic (era of aging of the population) changes.

One of the distinctions of development of our country is the absolute and relative growth in number of elderly people in the age structure of the population in general and in industrial groups in particular. This category of people is usually notable for high qualifications, great scientific-occupational and life experience [knowhow], as well as the ability to transmit the knowledge they have accumulated to the next generations.

The process of aging of the population of the USSR has put to society and science several new tasks related to employment and services for the elderly, development of optimum labor standards with consideration of age-related distinctions, study of the needs and capacities of the aging person.

From the economic point of view, it was interesting to make some estimates of the "reimbursement" from an elderly individual engaged in socially useful work. A comparison of the State's expenses on an elderly individual to results of his work would enable us to determine the extent to which it is expedient or inexpedient to employ individuals who have retired because of their age and who have retained complete or partial work capacity.

There are many reasons for retarded aging. We should mention here the increasingly broad and intensive use of advances in science and technology for the prevention and treatment of diseases, strengthening the defense mechanisms of the body, stimulating or replacing any "defective" organs, stricken regions, etc. At the same time, the entry of more and more countries and peoples on the road of democracy and socialism widens the social base for increasing the number of elderly people who have retained their health and work capacity entirely or partially.

The phenomenon of retardation of aging meets the new demands of a number of areas of human endeavor (science, services, public health, education, upbringing rising generations, etc.). The rapid pace of rise in number of elderly individuals is instrumental in satisfying, to some degree or other, the need for highly qualified manpower with abundant life experience and professional skill, caused by modern scientific and technological progress. Elderly people can make a substantial contribution to different sectors of the national economy, as well as to bring up rising generations, handing down the knowledge they have accumulated, provided they have retained complete (or at least partial) work capacity and there is scientific organization of labor.

Apparently, the increase in share of elderly and aged individuals in the age structure of the population of our planet must have some influence on reproduction and physical development of new generations. It is interesting (not only theoretically, but practically) to study the problems that are emerging, because of the need to effectively regulate and make optimum plans for relations between generations with consideration of both their social and biological needs.

In the presence of intensification of vital activities and expansion of the actual range of work capacity of older generations, society is faced with the task of making a serious revision of its attitude toward the final period of a man's life. Contemporary elderly people are becoming less and less suitable for the role of passive observers of events. They can and must remain as

active subjects and creators of social, scientific and technological progress. In this respect, the task of vocational rehabilitation of elderly individuals who have retained their work capacity acquires special humanistic significance. They should be used more (but, of course, on a voluntary basis and within reasonable limits) in solving national economic problems.

Apparently, the typical distinctions of the elderly, as well as their abilities and requirements, must be submitted to further scientific investigation and generalization in the most varied aspects, including determination of possible links and relations between development of certain types of thinking (for example, analytical or synthetic) and age periods in the life of an individual. Aside from its applied significance, research in the area of age-related biology and physiology of man and its interaction with his social traits could also serve for further deployment of theoretical investigations of the process of inception and development of personality.

A socialist society provides the best conditions for aging people, for their utmost useful activity and preservation of optimism in them. Measures of social security of the elderly are based primarily on recognition of the value of each human being, including the elderly. For this reason, bourgeois conceptions, which rate the process of demographic aging as regression of society, must be unmasked.

In a capitalist society, aging of the population generates new clashes, which are related to disruption of the balance between age groups. Antagonism among the elderly is manifested by an inadequate income and absence of meaningful social roles for some and increasing in buying power of others. This transforms the latter into a noticeable group of idle people who are pleased with themselves and leads to a new phenomenon in bourgeois society--gerontocracy.

Finally, the last of the trends mentioned is the shift in time of actual (biological) old age, its deferral to a more advanced age. There is every reason to believe that occurrence of actual old age will shift as society develops. This tendency is also manifested by later extinction of reproductive functions, which has shifted by more than 2 years in women in the last 100 years. As a result, the reproductive stage of the life of modern generations has been prolonged. This is also one of the consequences of sociohygienic, scientific and technological progress. But the existence of such a trend creates a discrepancy between juridical and biological time of occurrence of old age. This circumstance (as well as, incidentally, acceleration of physical maturation of young generations) makes it necessary to work out adequate and scientifically validated criteria of the actual limits of age periods of development of contemporary and future generations.

The tendencies we have discussed above can be assessed as being purposeful and, provided of course that appropriate beneficial conditions are present, they enable man to express the most fully his socially meaningful biological inclinations and potential (health, constitutional distinctions, type of nervous system, etc.). Earlier physical development of modern children and adolescents, and postponement to a later time the age of biological old age widen the reproductive period of human development, raise significantly the mean life expectancy of modern

generations in general and the creative period of their life in particular. Comprehensive and in-depth investigation of these trends requires that the efforts of scientists in different specialties and from different countries be united on a scientific and humanistic basis. Their objective should be to learn about and wisely regulate factors of evolution of the human body in the interests of development of man himself and society as a whole. Obviously, in this context there must also be maximum consideration of the capabilities of modern general social, scientific and technological progress, as well as active use thereof to further improve the human race.

Substantial changes are also observed in the dynamics of birthrate. The changes in birthrate are inversely related to industrial, scientific and technological progress and, in particular, to the number of women employed in the national economy. The more intensive industrial, scientific and technological progress and the more women involved in it, the lower the birthrate. This is illustrated by the differences in birthrate of regions differing in economic and cultural development. In areas with high economic and cultural development it is lower than in regions with less such development. As a rule, there is a higher percentage of women engaged in social labor in regions with a high level of economic and cultural development. Their further involvement in active participation in socially useful labor will require concurrent solution of the birthrate problem. The fact of the matter is that in the years of Soviet power the birthrate in our country has decreased to more than one-half. Before the revolution, there were 45 births per 1000 population, whereas the figure for recent years is 18-19 births. Some demographers believe that a further decline of birthrate cannot fail to affect not only the age structure of the population, but manpower resources of the country.

The increase in number of elderly people in the age structure of the population requires solving a number of concrete problems pertaining to medical care and employment. The increase of this group also requires more intensive development of such disciplines as gerontology and geriatrics.

Further progress of science and technology will be even more successful if society learns to control biodemographic processes, proceeding from the interests of man and mankind in general.

The existence of numerous diverse links between development of science, technology and biodemographic processes requires an increase in society's responsibility for proportionate, harmonious and purposeful development of all these elements, distinction and investigation of several directions of paramount importance that gravitate toward philosophical science, technological knowledge and biodemography. Synthesis of these branches of knowledge, which is made on a successively scientific and humanistic basis, will be important to further development of world view and methodological functions of Marxist-Leninist philosophy.

Maximum accumulation of knowledge about the potentials for development and reciprocal influence of science, technology and biodemographic bases of society, development of adequate methods and theories that take into consideration the possibility of reciprocal influence thereof, use of various models and a complex approach to the study thereof with due consideration of the existence of different types of social systems and value orientations could have a beneficial effect on further work on the problem under discussion.

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POSSIBILITY OF HYPERBARIC OXYGENATION AT MEDICAL EVACUATION STAGES

Moscow VOYENNO-MEDITSINSKIY ZHURNAL in Russian No 5, May 82 pp 16-19

[Article by S. B. Gatagov, Honored Physician of RSFSR, Maj Gen Med Serv, A. A. Rafal'skiy, Col Med Serv and A. F. Litavrin, Lt Col Med Serv]

[Text] The problem of effective control of hypoxia is of great interest to all military physicians. This is attributable to the fact that hypoxia not infrequently complicates such pathological states as combat trauma with wound infection, acute poisoning, damage to the respiratory tract by gunpowder fumes, toxic smoke and other highly toxic impurities (powder disease, thermochemical damage to respiratory organs complicated by toxic pulmonary edema, etc.).

It is a complicated task to organize medical care of this group of wounded and sick in a combat situation. Acute hypoxia will make it quite difficult to implement therapeutic and evacuation measures. Early use of hyperbaric oxygenation (HBO) at different stages of medical evacuation is necessary for prompt restoration of oxygenation of the body. As shown by the experience of peacetime, wide use of hyperbaric oxygen in the practice of medical institutions makes it possible to reliably shorten the period of patient treatment and avoid development of many dangerous complications.

HBO is the most effective in the treatment of individuals with acute carbon monoxide poisoning. For example, it was been proven that hyperbaric oxygen has high selective efficacy (even if drugs are not used) in cases of poisoning by carbon monoxide and nitrous gases. HBO therapy is particularly effective in combination with antidotes in cases of poisoning by cyanides and organophosphorus compounds (insecticides, metaphos, chlorophos and others).

In such cases, control of hypoxia is secondary in urgency, after giving an antidote and administering medical measures (V. D. Tonkopiy, 1974). When such poisoning occurs and antidotes no longer help in the presence of marked hypoxia, hyperbaric oxygen could be an irreplaceable means of high-speed treatment for victims.

The question of combined use of HBO and extracorporeal hemodialysis also merits attention. Our experience has shown that such a combination was highly effective in intensive care of some diseases complicated by decompensated hypoxia.

combat situation, the probability of carbon monoxide poisoning increases. As a result of inhaling powder (explosive) gases, hot air saturated with carbon monoxide and dioxide, as well as other toxic chemicals, military personnel could develop powder sickness and thermochemical lesions to respiratory organs associated with severe toxic pneumonia, pulmonary edema and acute respiratory insufficiency. Nitrous gases strike primarily the respiratory organs; they cause development of toxic edema of the lungs and methemoglobinemia. Most such patients are in the untransportable category. Without receiving prompt medical care, they could perish near the site of injury.

According to the data of V. A. Dolinin et al. (1976), the severity of the victims' condition is attributable to the multifactored nature of injuries, which is often the cause of high mortality at the site of injury, particularly in fires. Overheating lowers man's resistance to carbon monoxide and other toxic agents (A. A. Tiunov, V. V. Kustov, 1969). Such patients tolerate transportation poorly and for this reason require immediate HBO therapy near the site of casualties, at the earliest possible time.

The experience gained in departments (offices) of hyperbaric oxygenation at our medical [therapeutic] institutions revealed that HBO is the method of choice in controlling progressive hypoxia in cases of diverse poisoning and severe pneumonia with marked respiratory insufficiency. It is also used with success in cases of trauma to the brain and spinal cord, wound infection, etc.

V. M. Semenov et al. (1981) and P. T. Demchenko (1981) submit data on the efficacy of HBO in rendering emergency care to victims with extensive and deep skin burns combined with thermochemical damage to respiratory organs. Some patients (about 32%) presented predominant damage to respiratory organs. For this reason, it was necessary, first of all, to eliminate pulmonary edema, bronchospasm and acute respiratory insufficiency. The authors showed that application of HBO in the set of therapeutic measures made it possible to eliminate pulmonary edema within 10-12 h in 11% of the serious cases and to stabilize the condition of all victims in this group within 24-36 h. Sufficient knowhow has been gained at some military hospitals in use of HBO for treatment of suppurative and septicemic diseases, in the pathogenesis of which hypoxia is of some importance, and sometimes even of leading significance.

The medical service of the USSR Armed Forces has the necessary number of T/O mobile and permanent pressure chambers that are used for therapeutic purposes. The mobile recompression unit [station] (Figure 1) is designed to prevent development of the specific diseases of divers and to administer therapeutic recompression. In the chamber, the victim can use an oxygen inhaler or a mask for inhaling oxygen from a portable tank, which has a reducing valve. Compressed oxygen is delivered through a special sealed duct to the breathing equipment located in the chamber. The routes of delivery of oxygen and air into the chamber must be self-contained. The recompression unit is mounted on the chassis of an all-terrain vehicle with a trailer.

The tactical characteristics and specifications of the Irtysh-MT portable O_2 compression chamber (Figure 2A, B) enable us to recommend it for use at all stages of medical evacuation. In the course of special tactical medical exercises, we tested its capabilities with regard to transporting the chamber, reliability of operation thereof and we estimated the time required to perform different

operations. It was established that the average time required to prepare the chamber for use is 10 min; 20 min are needed to raise pressure in it to the operating level and 30 min to raise pressure to this level in an emergency; 90 min are needed for the chamber to function in a self-contained mode and up to 1.5 min for emergency pressure lowering. Five min are needed to prepare the chamber for transportation. Use of the chamber in therapeutic institutions demonstrated its good therapeutic capabilities (I. N. Bukhalovskiy et al., 1975; Yu. G. Shaposhnikov, B. Ya. Rudakov, 1975; V. I. Gerasyutenko, V. I. Gurov, 1981, and others).

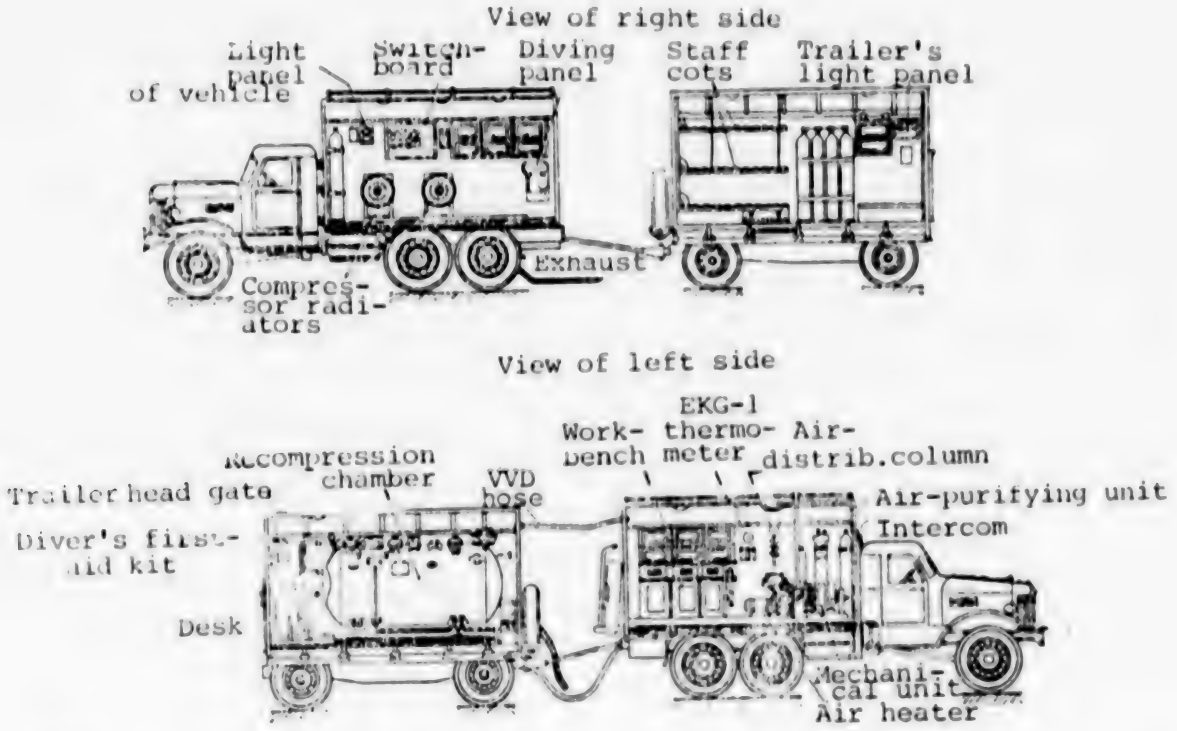

Figure 1. Mobile recompression unit (sketch)

The single-patient therapeutic Oka-MT pressure chamber (Figure 3) is generally used at a hospital. Oxygen is supplied from the oxygen system or a tank at a specific pressure.

The flow-type PDK-2 decompression chamber is stationary, and it can accomodate two stretcher cases or four sitting up. Our experience with PDK-2 for therapeutic purposes is indicative of a need to install an additional air filter, pneumo-inhalator and pneumatic suction pump, bactericidal lamp, RPA-2 unit, two-way nonsparking communication device and others. The required diagnostic equipment was connected by special airtight ducts. A special device we designed turned out to be very convenient for transporting serious cases into the chamber: the patient lying on a standard stretcher is placed on a "couch" in the treatment compartment, which can be moved by means of a removable connecting frame over the special tracks for stretchers.

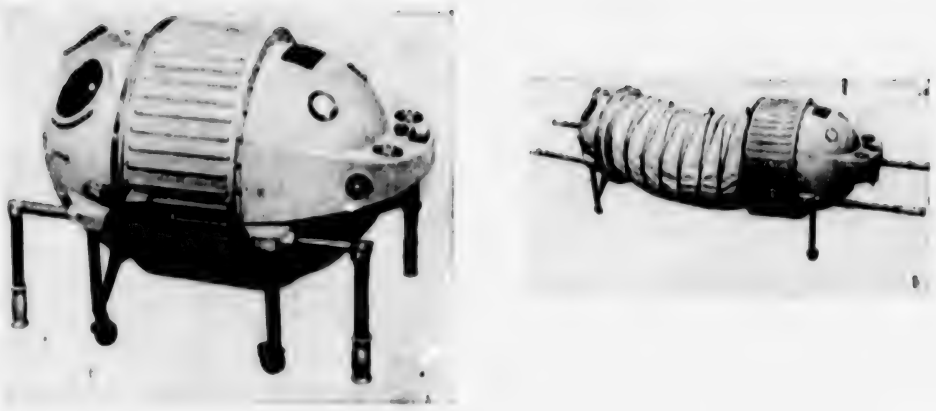

Figure 2. Irtysh-MT portable oxygen compression chamber

A) prepared for transportation B) prior to HBO session

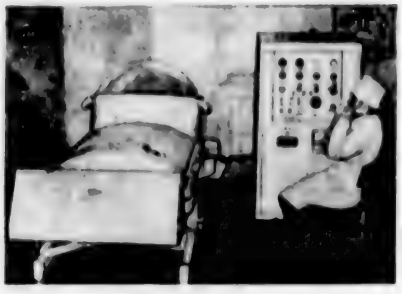

Figure 3. Individual therapeutic pressure chamber

For many years we have already used the above-mentioned recompression chambers for medical back-up of dives (P. A. Pal'chikov et al., 1981), therapeutic recompression in cases of caisson's disease and barotrauma to the lungs, as well as treatment of patients (G. A. Vilyunov, Yu. N. Osherovskiy et al., 1973; I. A. Sapov et al., 1975; A. F. Litavrin et al., 1981).

According to the data of a number of military medical institutions, about 25% of the patients are submitted to hyperbaric oxygenation in accordance with vital signs. Use of HBO in rendering emergency care to victims at the site, as well as in conjunction with other therapy for patients with various diseases, at the Central Military Hospital of the USSR Ministry of Defense, okrug and other military hospitals (Baltic Military Okrug) showed it to be highly effective. For example, in cases of barotrauma to the lungs and caisson's disease, all victims recovered. HBO therapy was successful in 89% of all cases

of diseases of vessels of the extremities, 88% of serious and terminal states, 76-83.3% of the cases of various types of poisoning, 80% of anaerobic infection cases and 66.5% of cases of posthypoxia coma. In addition, HBO makes it possible to correct certain homeostatic parameters of individuals with chronic hypoxic states, as well as those who have worked for a long time with industrial liquids (V. I. Gerasyutenko et al., 1981; V. I. Gurov et al., 1982).

Mobile recompression chambers are also used extensively in the armed forces of some foreign countries in conjunction with other forms of early emergency therapy (Larcan et al., 1967). About 20 years ago, Smith proposed HBO use in small, mobile individual pressure chambers for cases of poisoning, and this subsequently made it possible to bring qualified medical care closer to victims.

The foregoing warrants the assumption that the method of hyperbaric oxygenation in wartime could become an essential element of intensive care of serious combat trauma, poisoning, life-endangering states, whereas mobile recompression units will be used directly at the advance stages of medical evacuation. This is also indicated by the medical exercises conducted in some branches of the USSR Armed Forces, military okrugs and experience with pressure chambers under field conditions.

In our opinion, portable and stationary decompression chambers can be used effectively in regimental medical stations, medical battalions, military hospitals, medical institutions of hospital bases and, in some cases, to transport casualties to another stage of medical evacuation without interrupting HBO. It is expedient to have stationary pressure chambers in medical institutions in the rear, to which casualties can be rapidly delivered by air ambulances.

According to peacetime experience, HBO therapy is indicated primarily for individuals suffering from diseases associated with decompensated hypoxia. They include poisoning by carbon monoxide and dioxide, injury due to corrosive fluids, toxic smoke and powder gases, casualties with toxic edema of the lungs. HBO should be prescribed for patients with serious mechanical trauma and burns complicated by shock and wound infection, with anemic hypoxia due to massive loss of blood, trauma to the brain and spinal cord, acute insufficiency of cerebral circulation, prolonged compression syndrome, etc. The number of such casualties could be considerable in a modern nuclear missile war, if the imperialists unleash one. For this reason, in our opinion HBO therapy in conjunction with other measures of qualified and specialized medical care would definitely be effective and improve the outcome of treatment of the wounded and sick.

According to data in the literature, the efficacy of hyperbaric oxygenation is related not only to the time that has elapsed from the moment an illness started (poisoning, trauma, etc.), but to the stage of the pathological process. Our experience has shown that good results are obtained only when HBO therapy is prescribed promptly and at the early stage of disease, and not when numerous attempts to eliminate hypoxia by other means have failed. For this reason, treatment of patients in the pressure chamber should be started at the very earliest time. In a combat situation, this can be done provided this form of medical care can be brought closer to the sites of casualties.

Now, further meticulous elaboration of the principles of HBO therapy by means of mobile and stationary high-pressure chambers, comprehensive testing of these principles in various special tactical medical exercises in peacetime are a basic key of successful use thereof in a combat situation at different stages of medical evacuation. Broader use of the capabilities of HBO in treatment of different categories of patients will enable us to accumulate the necessary knowledge and train a sufficient number of specialist physicians.

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PREVOYAGE TRAINING CLASS FOR SHIP'S PHYSICIANS

Moscow VOYENNO-MEDITSINSKIY ZHURNAL in Russian No 6, Jun 82 pp 18-20

[Article by P. I. Korchagin, Col Med Serv, and A. S. Makaryshin, Lt Col Med Serv]

[Text] In modern times, the requirements have been raised with regard to physical condition of personnel and, accordingly, professional training of ship's physicians. The distance of cruising regions from bases virtually rules out the possibility of concrete assistance to ship's physicians by specialists of naval medical institutions and makes it difficult to evacuate the sick to a hospital. These circumstances make it imperative to render qualified medical care and some types of specialized medical care aboard ships and submarines at sea.

The ship's physician must be a broad specialist and have well-developed clinical orientation of the mind. He must not only implement therapeutic-preventive and epidemic-control measures, as well as see that sanitary and hygienic requirements are met on board, but have certain theoretical knowledge, skills in surgery and therapy; he must be familiar with allied branches of medicine, he must be able to use all of the modern diagnostic and therapeutic equipment provided on ships.

The purpose of internships for naval medical personnel is to have their participants--young physicians, graduates of the Military Medical Academy imeni S. M. Kirov and military medical faculties at medical institutes--retain firmly acquired knowledge in a relatively short period of time, acquire the practical skills necessary for independent work in the course of autonomous missions. For this reason, the problem of intensifying and optimizing the learning process through internships is a particularly pressing one.

We are devoting much attention to upgrading the existing syllabuses for training ship physicians, defining them better with due consideration of the specifics of medical support of ships during long voyages. We draw upon regular [T/O] instructors, chief medical specialists of the navy and the most experienced physicians for this work.

Under difficult conditions, complicated problems can be solved independently and decisions followed through without qualified assistants only if the ship's physicians are comprehensively trained. In order to perform this task we have worked out a roster of the main practical skills in each

specialty, started to keep a log of clinical training of the head of a ship's medical service (unit--chast') and established a class for prevoyage training of ship's physicians.

The purpose of the class ensues from its name, although it should be called, more precisely, the ship's physician class. The equipment for the class consists of displays with lists and samples of documentation for the head of the medical service of a ship and submarine, administrative documents, educational and reference medical literature, methodological instructions and guides of chief naval medical specialists dealing with emergency care during an autonomous cruise.

In view of the limited space, ship-board special-purpose medical equipment is represented by schedules [inventories]. The sets of surgical instruments for the most common emergency operations (appendectomy, tracheostomy, thoracotomy, venesection), packages of sterile surgical dressing materials and disposable linens are mounted on a separate display. There are also mock-ups and photo displays of shipboard walk-in offices, infirmary, surgery and isolation ward.

For development of skills in anesthesiology and resuscitation measures, anesthesia and resuscitation equipment and appropriate instruments have been installed and are used: Narkon-P anesthesia unit, portable manual artificial respiration unit and Gornospasatel'-8m [mountain rescue unit], laryngoscope, set of intubation tubes, portable surgical electric suction unit, tanks with oxygen and nitrous oxide and a Risasi-Anna training dummy.

A list of the drugs required for a voyage is furnished in the inventory of the emergency therapeutic care cupboard, which was outfitted with consideration of cruising experience and approved by the naval chief internist. There is a Malysh electrocardiograph for training in taking electrocardiograms.

In some cases, ship's physicians must know how to use portable x-ray units, which are represented in our class by the Arman-1 unit with instructions on how to operate it and set of typical x-rays. There are also installed physiotherapy units: for ultrahigh-frequency therapy, mercury-quartz lamp, diadynamic [?], Solluks lamp. It must be stressed that all of the equipment is in working condition and used actively in the course of the practical classes.

The locker for blood transfusions is outfitted with sets of sera, systems for taking and transfusing blood and solutions, vials with preservatives, instruments, etc.

The equipment of the class also includes chests [packages] for rendering medical care to individuals with ophthalmological, dermatology and ear-nose-throat diseases, as well as laboratory tests. There is a place ["corner"] for a sterilizer, rescue "evacocontainer" [litter?] with photo display, diver's gear and aqualung. The "shipboard operating room," which is used for training in preparing for surgery in a shipboard walk-in facility, is set up separately.

Practical lessons with students enrolled in different cycles are held in the prevoyage training class. Here, they can make a detailed study of documentation, shipboard medical equipment in action, consolidate their theoretical knowledge and practical skills, which are needed for a long-term self-contained voyage. In our opinion, classes for prevoyage training of ship's physicians must be organized during the internship of all naval medical personnel.

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ORGANIZATION OF INTENSIVE CARE IN A MILITARY OKRUG HOSPITAL

Moscow VOYENNO-MEDITSINSKIY ZHURNAL in Russian No 6, Jun 82 pp 20-23

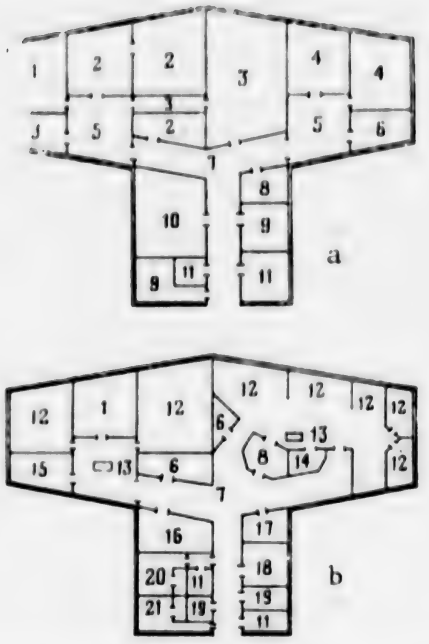
[Article by V. N. Kharlamov, Honored Physician of Latvian SSR, candidate of medical sciences, Col Med Serv, V. S. Palagin, Col Med Serv, I. A. Pleskachev, candidate of medical sciences, Col Med Serv, F. I. Untu, Col Med Serv, Ye. A. Litvinko and A. M. Reventsov]

[Text] The last decades have been characterized by intensive development of anesthesiology, resuscitation and intensive care. The organizational aspects of this process have been covered extensively in the special literature (M. I. Kuzin, A. A. Bunyatyan, 1971; B. V. Petrovskiy, 1976; G. N. Tsybulyak, 1976; V. A. Negovskiy et al., 1977; V. R. Chernyakhovskiy, 1979, and others). However, some questions dealing with organization of intensive care for the seriously sick at major military hospitals require further investigation.

Our 3-year experience in rendering intensive care in an okrug military hospital enabled us to demonstrate the advantages of treating patients there who are suffering from serious forms of various diseases and to draw some conclusions.

The anesthesiology and resuscitation complex established at the hospital (see Figure, a and b), combines the following in accordance with territorial characteristic [?] and functional purposes: departments of anesthesiology and resuscitation, hemodialysis, hyperbaric oxygenation, procurement and transfusion of blood, high-speed diagnostic test laboratory, emergency operating room and resuscitation wards. It also includes an x-ray laboratory. The complex is situated in the immediate vicinity of the admission department and emergency surgery department.

The department of anesthesiology and resuscitation, which has 15 intensive care beds (Figure, b), occupies an area of 400 m^2, 40% of which is referable to ancillary rooms (sterilization, utility, instrumentation, employees' room and others). There is an area of 12 to 18 m^2 per bed. A system for centralized delivery of oxygen, nitrous oxide, vacuum to each patient, as well as a system for grounding electric equipment in accordance with the instructions for safety practices. In addition, the facilities have been "shielded," for the purpose of precluding interference when operating diagnostic equipment. There is telephone communication between the department and other functional units of the hospital, as well as television-telephone communication with the examination post [traffic control?] so that patients can communicate with relatives.



Anesthesiology-resuscitation complex at hospital

1) resuscitation wards
2) hemodialysis department
3) hyperbaric oxygenation department
4) emergency surgery operating room
5) preoperative prep room
6) room for equipment
7) hallway
8) employee's rooms
9) department for procurement and transfusion of blood
10) high-speed laboratory
11) ancillary rooms
12) intensive care wards
13) nurses' station
14) medication
15) resident's office
16) sterilizing room
17) utility [dishwashing]
18) office of department head
19) linen room
20) employees' room and shower
21) food dispensing room

The combination of open and decentralized systems of laying out the intensive care wards makes possible constant visual supervision and monitoring of a specific category of patients, as well as to isolate patients referable to different types of diseases in order to prevent intramural infection.

Ultraviolet light is used regularly in the department, and the proper sanitary and hygienic conditions are maintained. An optimum microclimate is provided with constant circulation of air by means of air-conditioners and ventilation systems. Modern diagnostic test and therapeutic equipment is always ready for immediate use: electrocardiographs, spirograph, "radiocirculograph" [scanner?], thromboelastograph, portable x-ray machine, Astrup micro-pH-meter, system for continuous monitoring of various parameters of vital functions of several patients at a time, RO-5 and RO-6 positive-displacement respirators, Faza and Lada artificial lung ventilation units, Kholod-2F unit for craniocerebral hypothermia, defibrillators, cardiostimulators, etc.

Close contact has been established with diagnostic (functional diagnostics, roentgenology, laboratory and radioisotope) and therapeutic departments, so that the necessary therapeutic and diagnostic measures can be promptly implemented.

Specially trained medical personnel man the intensive care wards: 10 physicians specializing in anesthesiology and resuscitation (including two military officers), 18 anesthesiology nurses, 8 junior nurses for patient care and housekeeper-nurse. In addition to intensive care, the physicians and nurses of the department render anesthesiological assistance during operations, change dressings and participate in special examinations. In view of the fact that about 30% of the surgical interventions are performed under general anesthesia, the above number

personnel should be considered the minimum required to provide anesthesiological and resuscitation care, as well as intensive therapy.

We have organized around-the-clock duty for an anesthesiologist-resuscitation care specialist, who renders intensive care at night, as well as administers general anesthesia together with the on-duty anesthesiological nurse.

Two around-the-clock nurses' stations were organized in the intensive care wards. In the daytime, the patients are cared for by two anesthesiologist-resuscitation specialists, one of whom has surgical training and the other training in internal medicine, in addition to anesthesiological. The latter treats not only the general medical patients, but participates in treatment of surgical patients with serious concomitant diseases which, as a result of surgical intervention, anesthesia and infusion therapy, often present unique manifestations, causing diagnostic and treatment problems, even for an experienced internist without special training in anesthesiology and resuscitation.

However, in spite of the differentiated special training of resuscitation-anesthesiologists and the experience they acquired in treating seriously sick patients referable to different types of pathology, we consider it of exceptional importance for the patients to be seen daily, jointly, by the chief surgeon or internist, heads of special departments and attending physicians. This maintains a high level of specialization in diagnosing and treating the most serious cases, it is instrumental in rapid professional growth of physicians and reduces to a minimum the need for extensive consultations.

To determine whether patients should be put in the intensive care and resuscitation department, we adhere to the following rules: we admit in this department all serious cases for which there is even a slight hope of successful treatment; we do not refer patients there whose cure is hopeless, as well as those known to be infectious; we immediately transfer to a special department patients who no longer need intensive care rendered by anesthesiologist-resuscitation specialists.

The indications for treatment of patients in the anesthesiology and resuscitation department conform essentially to the recommendations of V. A. Negovskiy et al. (1977): acute cardiac insufficiency, pulmonary edema, acute cardiac arrhythmia, acute impairment of peripheral circulation (collapse, traumatic shock, hemorrhage, cardiogenic shock, recurrent acute myocardial infarction); acute respiratory disturbances; acute impairment of metabolism, acid-base equilibrium; acute renal and hepatic insufficiency; comatose state; severe poisoning; recovery period following agony and clinical death; need for prolonged parenteral feeding; serious conditions following extensive surgical interventions. The last group of patients constituted the bulk of cases treated in our department.

The decision to hospitalize a patient in the anesthesiology and resuscitation department is made following a mandatory examination of the patient by the appropriate specialist, together with the department head or on-duty anesthesiologist-resuscitation specialist. The diagnosis is pinpointed; the most warranted plan of therapy is worked out and determination is made of the special department in which the patient will be treated subsequently.

Resuscitation measures are administered to patients and casualties delivered by ambulance in a terminal state in a specially equipped resuscitation ward situated in the immediate vicinity of the admission department. This enables the hospital's on-duty physician to call upon not only the personnel of the department of anesthesiology and resuscitation for immediate participation in diagnosis and treatment, but on-duty personnel of the receiving [admission], emergency surgery and other departments, as well as consultants, if necessary.

In our opinion, it is unwise to put a patient who is in a terminal state immediately in the department of anesthesiology and resuscitation, particularly at night, since in such a case all the rather difficult diagnostic and therapeutic problems must be solved by the small number of on-duty staff of that department.

Individuals with diseases that require more definite diagnostication, emergency and complicated instrument tests (bronchoscopy, esophagoscopy, fibrogastroscopy), which are performed against the background of intensive therapy or general anesthesia, are also delivered to the resuscitation ward. After the diagnosis has been ascertained and the severity of the patient's condition is reassessed, further therapeutic tactics are determined and he is sent to the department of anesthesiology and resuscitation, a specialized hospital department or emergency surgery operating room.

A large group of patients suffering diverse types of pathology has been treated in our department over the 3-year period. We have noted that there has been a rise in number of patients in very serious condition. Mean duration of treatment was 4-5 days.

We use modern diagnostic and monitoring methods in the department, such as electrocardiography, electroencephalography, echoencephalography, thromboelastography, spirography, monitoring, determination of circulating blood volume, fluid-electrolyte and acid-base balance and others. Appropriate intensive care is rendered as indicated: intravenous infusions of drugs through a cava catheter, peridural postoperative anesthesia, intraaortic infusion of drugs, detoxification therapy, peritoneal dialysis, hemodialysis, hyperbaric oxygenation and others. All this, along with application of the above principles of organizing reception of patients in the department, has made it possible to lower the mortality rate.

The existing organization of intensive care of the seriously sick at a district military hospital has eliminated the need for individual medical stations, provided more time for the personnel of specialized departments to perform better patient examinations and administer better therapy; it has made it possible to use the anesthesiology-resuscitation complex as a base for training physicians and nurses of the hospital and other therapeutic institutions in the district in the area of anesthesiology, resuscitation and intensive care, including applications thereof to various phases of medical evacuation.

Thus, as can be seen from the submitted data, the advantages and, consequently, necessity of centralizing intensive care and resuscitation measures are unquestionable. At the same time, it is important to continue the search for reserves in order to further upgrade specialized medical care for the seriously sick at major military hospitals.

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USE OF ROSETTE-FORMING TESTS TO DETERMINE RADIOSENSITIVITY OF HUMAN LYMPHOCYTES

Moscow VOYENNO-MEDITSINSKIY ZHURNAL in Russian No 6, Jun 82 pp 63-65

[Article by Ts. Shchilik, Capt doctor of medicine, Ye. Krushevskiy, Capt doctor of medicine and V. Yendrzheychak, Major doctor of medicine (Polish People's Republic)]

[Text] One can determine in most cases the magnitude of absorbed dose from a nuclear weapon only on the basis of clinical symptoms. The main parameters of biological dosimetry of irradiation include intensity of decline in absolute lymphocyte, neutrophil granulocyte and thrombocyte content of blood (Dolphin, 1969; Kopec, 1971; Dziuk et al., 1976).

Considering the time of development of radiation sickness, a change in lymphocyte count is of greatest significance in diagnosing this disease. It is known that lymphocytes consist of cells referable to different subpopulations, which differ in life space and sensitivity to many deleterious factors. The main types of lymphoid cells are T lymphocytes, which are responsible for cellular responses (thymus-dependent) and B lymphocytes (thymus-independent, bursa-dependent) that are responsible for humoral responses.

At the present time, methods of quantitative assays of these cells in peripheral blood have been developed and are available. T lymphocytes are characterized by the capacity to produce rosettes with ram erythrocytes (so-called E rosettes), while B lymphocytes can form rosettes with the activated component of complement, joined by an antibody with erythrocytes--EAC rosettes (Jondal et al., 1972). A group is distinguished in T lymphocytes that forms active AE rosettes, whereas a subpopulation is present among B lymphocytes that forms rosettes with mouse erythrocytes--ME rosettes (Wybran et al., 1973; Woody et al., 1974; Dobozy et al., 1976; Potter et al., 1978).

It is interesting to find out whether these subpopulations differ in radiosensitivity and whether the radiosensitivity of some of them is more effective as an indicator of irradiation than the absolute quantity of lymphocytes. It was easiest to study radiosensitivity using in vitro cultures (Andrejczak et al., 1977).

There were 11 healthy male donors, 21-25 years of age, who participated in our studies. Lymphocytes isolated from their peripheral blood by means of centrifugation in a ficoll-uropolin density gradient (Boyum, 1968) were tested for viability by staining with trypan blue.

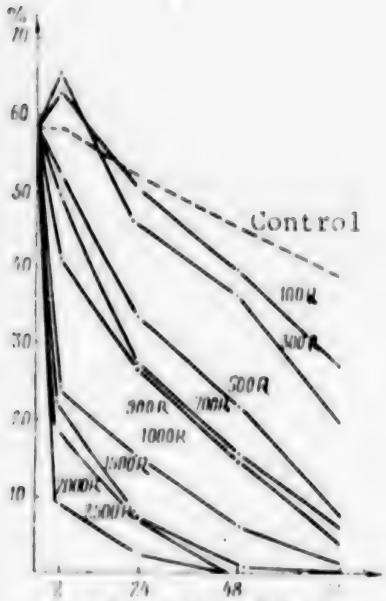

Figure 1.
Quantity of T lymphocytes forming E rosettes (percentage)

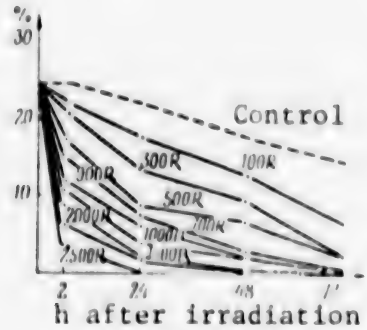

Figure 2.
Quantity of T lymphocytes forming AE rosettes

A suspension of cells was divided into two parts. One was exposed to x-rays and the other served as a biological control. The radiation doses ranged from 100 to 2500 R. Examinations were made 2, 4, 24, 48 and 72 h after irradiation. Suspensions that we planned to work with after 24, 48 and 72 h were placed in flasks containing the substances required to keep the cells alive and incubated at a temperature of 37°C. After irradiation, we counted lymphocytes per mℓ culture, determined viability of cells and examined some of their subpopulations for capacity to form E, AE, EAC and ME rosettes.

As can be seen in Figure 1, there was an increase in number of T lymphocytes forming E rosettes 2 h after exposure to doses of 100 and 300 R, after which there was a more drastic decline thereof than in the control culture. The difference was statistically significant starting at 48 h after exposure. Radiation in doses of 500 and 700 R did not cause a temporary increase in T lymphocytes forming E rosettes 2 h after delivery thereof. On the contrary, there were fewer such cells after exposure to both doses than in the control, and for the dosage of 700 R the difference was statistically significant. With a dosage of 500 R, the decline in percentage of cells forming E rosettes was significant starting at 24 h after exposure and it was the most reliable after 48 h. A rather reliable decrease in number of lymphocytes in this subpopulation was observed starting at 24 h after delivery of 700 R.

The number of T lymphocytes forming active AE rosettes dropped after the above doses of radiation already 2 h after exposure, and it was close to zero after 72 h (Figure 2). Already 2 h after delivery of 900 R, there was a decrease in quantity of T lymphocytes forming E rosettes. With doses of 1000 to 1500 R, the dynamics of decline were similar and, starting with a dosage of 1500 R, the number of such cells dropped to zero 72 h after exposure. Examination of T lymphocytes forming AE rosettes after delivery of 900 R was not part of our task, since we failed to demonstrate such cells in the culture.

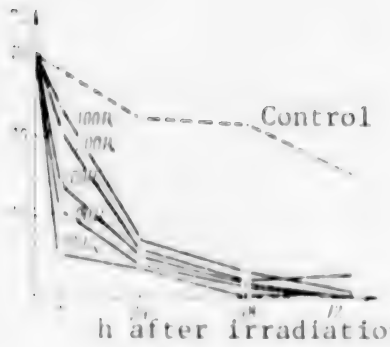

Figure 3.
Quantity of B lymphocytes forming EAC rosettes

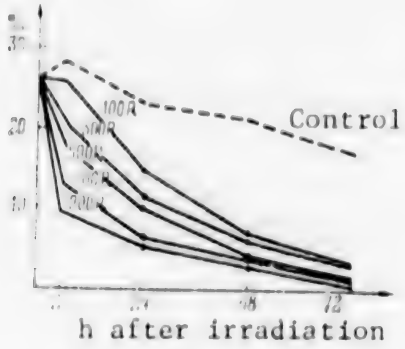

Figure 4.
Quantity of B lymphocytes forming ME rosettes

Figures 3 and 4 illustrate the dynamics of decline in number of lymphocytes forming EAC and ME rosettes. With radiation doses of 1000 to 2500 R we did not examine the number of B lymphocytes forming ME rosettes, since the dynamics of this subpopulation with lower doses were the same as of cells that form EAC rosettes. The quantity of B lymphocytes forming EAC and ME rosettes decreased proportionately to the increase in dose of x-radiation. The dynamics of decline in number of B lymphocytes, starting with a dose of 300 R, can be qualified as having two phases, with intensive decrease for the first 2 h and slow decline for the next 70 h.

On the basis of our in vitro studies, when resistance and viability of cells are much lower than in vivo, it can be stated that acute radiation in doses of 100 to 900 R does not elicit immediate death of lymphoid cells. In the first 24 h the quantity of viable cells diminished insignificantly, and this is consistent with the findings of other authors (Facchini et al., 1976; Kwan, Norman, 1977). The intensity of decrease in these lymphocytes increased proportionately to time and increase in dosage of x-radiation.

Isolation of several subpopulations among lymphoid cells, differing in function and presence of surface markers, enabled us to demonstrate very high radiosensitivity of the subpopulation of T lymphocytes that forms AE rosettes, as compared to the radiosensitivity of both subpopulations of B lymphocytes and general radiosensitivity of lymphoid cells.

Thus, these studies revealed that the absolute quantity of cells forming AE rosettes in peripheral blood is a considerably better indicator of irradiation than the absolute quantity of lymphocytes. Of course, it is simpler to determine the absolute quantity of lymphocytes, but the AE test is also simple and can be done in any laboratory where the absolute quantity of lymphocytes is counted. This test does not require complicated reagents, and one laboratory technician can run 24 analyses in 4 h. This means that this test can also be performed in the laboratory of a field hospital. It is also desirable to use this test when there are inadequate records to substantiate exposure to radiation, as well as in cases of radiation accidents.

Prosser (1976) and Facchini et al. (1976) observed that B-lymphocytes forming EAC rosettes demonstrated somewhat greater radiosensitivity in vitro than T-lymphocytes forming E rosettes. Dobozy et al. (1976) and Potter, Moore (1978) report almost identical changes in quantity of B lymphocytes forming EAC and ME rosettes under the influence of ionizing radiation.

Thus, we should stress the great importance of rosette-forming tests, particularly the AE test. The high sensitivity of the test and relative simplicity of performing it warrant recommending it for diagnostic purposes to detect acute radiation sickness, including its use at different stages of medical evacuation.

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BURENKOV ON SOVIET PUBLIC HEALTH CARE

Moscow LITERATURNAYA GAZETA 21 Jul 82 p 12

[Interview with USSR Minister of Health S. P. Burenkov by LITERATURNAYA GAZETA correspondent Anatoliy Rubinov; data and place not specified: "The Health of the People"]

[Text]
- Why when there are so many physicians do we sometimes feel a shortage?
- When should polyclinics be open?
- Can a pharmacist treat patients?
- Why should an electronic computer know all about men's hearts?

A. Rubinov: Sergey Petrovich, in the last year our country's many medical institutions started experiencing some new trends. There was a definite revitalization. The winds of change blew and at many levels a strong tendency to listen to criticism is becoming apparent. Could you briefly describe the fundamental task that the ministry is setting before the medical professionals? And, most important, what about it is new?

S. Burenkov: First of all, I must say that the Ministry of Health is responsible to the party and the government for the level of medical care in the country, and the ministry's work is determined by the decisions of the 26th Party Congress and by the critical observations made there by Leonid Il'ich Brezhnev. A 1977 resolution, "Measures for Further Improving the Health of the People", made by the CPSU Central Committee and the USSR Council of Ministers, plays an important role and serves as a program document for the Ministry of Health. You are asking me, however, to describe what is new about our program. What is new is that we are directing our attention toward the initial stages of the health care process. Every person who gets sick turns first to a polyclinic or to an emergency medical service. That is where treatment begins. A great deal depends on how quickly and accurately the diagnosis is made, and on what treatment is prescribed. Primary health care involves rural health institutions and medical-health care units in enterprises and institutions. New, extremely important tasks for improving

the medical care available to our rural populations were outlined by decisions made at the May (1982) Plenum of the CPSU Central Committee. The primary concern is bringing medical help closer to village residents.

A. R.: Our country has the highest number of physicians in the world. Recently some figures were published showing that at the end of 1980, there were 995,600 physicians.

S. B.: Since that time, there has been another class graduating from medical schools. I can offer some newer data. We now have 1,028,000 physicians--that's more than 38 for every 10,000 people.

A. R.: And how many do we need? Why is it that with so many physicians, we sometimes feel a shortage?

S. B.: We must consider the peculiarities and prospects of Soviet health care. We set ourselves the task of preventive health care, so physicians also see and examine healthy people. In addition, medical services at industrial enterprises have been developed extensively. Railroad workers and aviators also have their own polyclinics and hospitals. We have a large network of pre-school institutions which have their own physicians. The same is true in the school. No small number of physicians are working in physical education and sports. As a matter of fact, here again we are talking about preventive care--we are keeping healthy people healthy. Finally, we have many scientific medical institutions with high concentrations of specialists who are also directly involved in medical work. To give a more complete answer to your first question, I would like to say that we see an urgeat need to increase the effectiveness of the work in our polyclinics, and we have set ourselves the task of organizing consultaiton-diagnostic clinics along with our regular, territorial, clinics. These must be bulit up from a foundation of medical institutes and multi-departmental clinical hospitals. Our primary goal is to attract highly qualified specialists to patient treatment at the pre-hospital stage. It is important that we be able to attract the best professionals to be involved at the very onset of an illness--in the necessary cases!

A. R.: Sergey Petrovich, would it be possible for me to make one overdue critical comment? Not so much for the sake of the comment itself, but really to get something off my chest.

S. B.: Doesn't LITERATURNAYA GAZETA usually make critical comments without asking permission? Your paper has carried so many articles critical of our health care!

A. R.: It is just one of those articles that I want to discuss. In 1968, not long after the national economy switched to a five-day work week, LITERATURNAYA GAZETA came out in support of polyclinics' maintaining a schedule of working six full days. That was the essence of an article by the writer Yanovskaya entitled "Let the Physicians Rest--but Not Medicine." Forgive this overdue reproach, but at that time the ministry paid no attention

to the article, even though hundreds of readers supported the newspaper's position. Exactly five years later, LITERATURNAYA GAZETA permitted itself to play a little trick. It published the same article, under the same title, without changing a word. The problem turned out to be just as urgent. Sergey Petrovich, how do you feel about this question? It does have a direct relationship to raising the level of work at the initial stage of the health care process.

S. B.: Certainly, we place a great deal of importance on polyclinics' work schedules. Two meetings of the republic health ministers were devoted to this question, and it was discussed exclusively. We believe that all specialists should be working in polyclinics on Saturdays. It is true that on Saturdays, as many years of experience have shown, fewer people visit the polyclinics.

A. R.: They simply got out of the habit.

S. B.: That is entirely possible. But since Saturday has turned out to be a day when people have more free time, we would like that to be the day for them to come in for treatment. On Saturdays people don't have to rush off somewhere, as they do on hectic workdays. We would like polyclinics to invite people to come in for health examinations on that calm day. I should mention that this is already being done in Latvia, and it is going well. We have had to straighten out the ministries in several republics, however.

When we discovered that some polyclinics had started working from 10 in the morning to 6 in the evening, we required them to change their schedule. For example, we had some sharp criticism for the Georgian Ministry of Health a year and a half ago. Conditions are supposed to be set up everywhere so that people can come to a polyclinic any time of the day--from 8 in the morning until 8 in the evening. A person anywhere should be able to meet with a physician without having to miss work; he should be able to turn to a physician not after he has already become ill, he should be able to come simply for advice.

I would like to say a little more: some polyclinics, those with extended hours, are supposed to receive patients on Sundays also, although in this case it is important to consider local conditions, possibilities, and customs.

There are no polyclinics operating only five days per week! If this schedule is still encountered somewhere, it is a flagrant violation of the ministry's requirements. The title of that article of yours was correct: Let the physicians rest--but not medicine. Physicians and nurses can have two days off per week--in the large institutions. Those places should use a rotation plan. But this will not work at the smaller institutions. The head physician and other staff must work six days a week.

A. R.: And what kind of weekly schedule should hospitals have? Is a six-day work week really sufficient? Everyone who has ever had a long stay in a hospital knows how quiet it is there on Saturdays, and on Sundays life comes

to a standstill. On Saturdays they perform only those procedures which cannot be delayed. On Sundays as well only the most seriously ill patients receive treatment. The others just get a "rest". A rest from treatment! They wait despondently for Monday, when life will resume. The result is that a patient's recovery is put off by another two days. Imagine how much people lose this way, and what kind of emotional upset it costs them! And think how much the government loses if a person's recovery and his return to work take an extra 8-10 days in a month!

I understand how important it is for medical workers to relax together. Everyone has a family. But really, isn't it possible to work things out so that the treatment process can continue uninterrupted?

S. B.: Hospitals are institutions in which the treatment process does continue uninterrupted. Here it is simply necessary to shorten the patient observation periods and carry out treatment in the proper amount of time, with no interruptions. No one is interested in having people spend a long time in the hospital. In some of the country's larger hospitals, we are now conducting an experiment directed at intensifying the physician's work. The basis of the experiment is a rational organization of the entire treatment-diagnostic process, in which the patient is quickly examined, with no delays, and treatment is started without delay. The entire process becomes more efficient. It is true that this required changing a number of financial standards. For a long time our expenses have been calculated on the number of beds occupied. But what are we treating, really, beds or people?

We would really like to see this experiment conducted in an energetic, yet careful manner. We have many excellent hospitals--in Minsk, Kiev, Moscow, and in large cities in Siberia and the Urals--where the treatment process is organized with no delays of any kind. But there are also hospitals where everything is done as if in a slow-motion movie. No, we don't need a feverishly-paced system of activity--not under any circumstances! The hospital day must be organized and full, so that the patient doesn't waste any time there. We are also firmly against discharging patients before medical indications allow, even if it is just one hour early.

A five-day or six-day work week at a hospital? Hospitals operate continuously, around the clock. But in hospitals Saturdays and Sundays do differ from regular weekdays. This is justified sometimes, however. For example, the operating room cannot be used every day. The technology simply does not allow it. It is not necessary for the operating room to be used all the time, except in hospitals providing around-the-clock emergency surgery. As far as physical therapy and other medical units are concerned, they should make better use of their resources. We are now taking energetic steps in that direction.

A. P.: The LITERATURNAYA GAZETA editors have always received many letters in which our readers discuss questions of health care. We send the majority of this flow of letters to the USSR ministry and to the republic ministries. How are the letters handled there?

S. B.: We give them our utmost attention. It is very important for us to know how our patients feel about the work our system does. Recently, we stopped being content with just passively waiting for opinions to come in. There is now a new form of interaction between medical institutions and the population: polyclinics are evaluated in front of residents of a microrayon. Last year 60,000 such evaluations were conducted. And that really isn't very many! I believe that there should be twice as many such meetings, with the head physicians present. They have turned out to be very useful. The citizens pose new questions and make critical observations. This enables us to notice things that for some reason we had missed before.

A. R.: Has the term "corridor patient" come up at these meetings between physicians and the population?

S. B.: Yes, of course.

A. R.: You won't find that combination of words in any reference book, but everyone knows what it means.

S. B.: We are doing everything we can so that term will be forgotten, so that it will become an entry in some reference book of forgotten terms. I have already mentioned our efforts to raise the level of organization of hospital treatment. I have no doubt that all this will give us the opportunity to treat a greater number of people. We have set a task for ourselves; no more corridor patients! As of now!

A. R.: But is that really possible? Is the hospital network developed enough?

S. B.: Theoretically there are enough hospital beds, that is, according to the standards for 1,000 or 10,000 residents. But there are cities where these standards should be higher. For example, many people want to be treated in Moscow. In the capital there are many, many out-of-towners who want to have consultations with well-known specialists, and to be examined in the famous institutes. There are cities where more facilities need to be built. But rather than talking about the actual number of spaces in hospitals, it would be better to discuss a more rational, better thought-out utilization of the beds we do have.

A highly organized medical system should lead to hospitals that are able to treat more people. Then we won't have any corridor patients, there will be no more unfounded rejections for hospitalization. There are, of course, circumstances in which someone can be admitted to the hospital today, or in two weeks. But there are so many cases in which delays are not possible! And in such cases, a rejection is no longer just a violation of the rules, it is a crime.

A. R.: Recently high hopes have been placed on total preventive health care for the entire population.

S. B.: This is a very important question. It warrants more detailed discussion. We consider our most important task to be perfecting our preventive work. And total preventive health care is just what I am talking about. It must be expanded and organized. Our scientists have done a good job laying the scientific foundations for primary preventive care, especially in cardiovascular diseases. These diseases are now the major cause of death. Applying scientific developments in practice opens the way for early detection of developing hypertension and ischemic heart disease, which in turn allows us to lower the incidence of serious complications of these diseases, such as acute myocardial infarction and stroke.

A. R.: According to materials from the World Health Organization there has been a general rise in the incidence of cardiovascular diseases in some countries recently, but at the same time, the number of deaths from these diseases has dropped.

S. B.: Some new figures that we find very encouraging have just been released: deaths due to cardiovascular diseases have declined in our country for the first time in several years.

A. R.: Can you say anything about a trend?

S. B.: We'll have to be cautious and patient, and see what the figures for 1982 show.

A. R.: But there are already cities in which preventive care has had excellent results. I had the occasion to write about Kaunas and the work of the academician, Zigmas Ippolitovich Yanushkyavichus. He initiated and directed a program in which all the men in Kaunas had their electrocardiograms recorded on a computer. The men were done first because heart disease affects the "stronger sex" more often. The computer also helps remind absent-minded people when it is time to visit the physician. Everyone has a specific interval between visits that depends on the electrocardiogram results and on the individual's state of health.

S. B.: I am very familiar with Academician Yanushkyavichus' work. Last year I visited him and had a close look at his laboratories. I believe that the preventive work being conducted by his collective on a city-wide basis is of great practical importance. This is precisely the path that Soviet health care must follow. It promises a large decline in deaths due to cardiovascular diseases.

But Kaunas is not our only example. We are proud to say that in recent years cardiology services have been organized on a country-wide basis. This is a streamlined, efficient system. At its head is the All-Union Cardiology Scientific Center, and in the republics we have a total of 14 scientific centers. There are hundreds of cardiology departments in hospitals and not just one thousand, but thousands of cardiology units in polyclinics, and this is very important. Utilization of these services in primary preventive care allows us to achieve modest, yet encouraging, results.

A. R.: A good deal is written in the foreign press about the growing death rate among children in our country.

S. B.: Those reports are not accurate. For many years the death rate among children here has declined systematically. There is an indicator called the index of healthy children. It is arrived at through a complicated series of calculations. This index is also improving. Nonetheless, one of the most important jobs for health care agencies remains the protection of the health of mothers and children. We still have many resources for improving the work of medical institutions, as well as children's and preschool institutions. The main thing here is of course also preventive care. Children's illnesses should be less frequent and less serious. Children's vaccinations should be developed further.

A. R.: The opposite situation has occurred with the vaccination, however. It is no longer necessary to vaccinate against smallpox, since it has disappeared.

S. B.: Yes, smallpox has been conquered. The international war against smallpox has had a successful outcome all over the world. It was conducted within the framework of the World Health Organization, and incidentally, was the initiative of the Soviet Union. We took a very active role in the process. The Soviet Union provided more than one billion doses of anti-smallpox vaccine to help realize the war against smallpox.

A. R.: Are there any other vaccines that you expect to become unnecessary?

S. B.: Not yet.

A. R.: Sergey Petrovich, when speaking of the primary stage of health care, you mentioned emergency medical services along with the polyclinics. Do any standards exist for the "Skoraya pomoshch" [the emergency medical service] that goes out on calls?

S. B.: Yes, of course. Naturally, they vary from city to city. They are different for large, middle-sized, and small cities. In many cities these standards are well-maintained, that is, the work is clearly organized and disciplined. A lot depends on organization. For a long time, the emergency medical service in Kemerovo had an unsatisfactory record. A thorough examination of the reasons for this uncovered that the service had no need of additional vehicles or additional personnel. Now the Kenerovo emergency medical service is running efficiently.

I didn't bring up Kemerovo by accident. It is a big industrial center, with a large population. Of the large cities, I would like to mention Kiev, Vilnius, and Leningrad as places with well-organized emergency medical services.

A. R.: And how about Moscow? Our editors have the feeling that the capital's emergency medical service has begun to operate better also. In any case, complaints about the service have stopped coming in.

S. B.: We would have to include Moscow among the best emergency services, although during the first days of the new year there were some problems. We investigated and managed to work things out within several hours. Complaints to the ministry about the Moscow emergency medical service are also coming much less frequently now. But we understand that not everything has been done yet. We realize that Moscow is a huge city, both in population--8,300,000 residents--and in area. The work of all the links in the chain of this crucial service is especially important in Moscow. We are gratified that the improvements have been so noticeable.

A. R.: Sergey Petrovich, on behalf of the LITERATURNAYA GAZETA readers, I would like to ask you two more rather pointed questions. Both have been discussed publicly. One of them has been discussed a great deal. Our editors are constantly receiving letters concerning the shortage of medicines. Some of the republic health ministries decided to grapple with the medicine shortage in a rather strange way: they prohibited physicians from prescribing substances that were not available in the nearest pharmacy. The pharmacy, however, may not be simply out of the scarce medicine. It could be that some shipping clerk or supplier is not doing his job, he may be loafing, and the physicians are helping him out by employing this dangerous practice. The result is that in some places, the patients have been assigned to a specific pharmacy. You can buy bread and produce in any store, but sometimes medicine can be obtained only at the assigned place.

S. B.: This is a disgraceful situation. The republic ministry's order was wrong, and the USSR ministry has made them change their directive. A new system has been in effect since July 1. There is definitely a shortage of some medicines, even though in every five-year plan the quantity of medicines going to pharmacies increases almost 1 1/2 times. That's every five years!

The demand for medicines is not the same everywhere. This can be explained by the differences in the make-up of the population. In those republics with a larger population of older people, more medicines are used. Sometimes the difference is quite large--30-40 percent.

The shortage would not be felt as sharply if physicians were more familiar with new products. To correct this we did the following, starting in Moscow: we opened pharmacological information offices in polyclinics. Pharmacists inform the physicians about the preparations they have received, including new ones that are analogs and can be used interchangeably with others, since they have the same effect.

A. L.: But people love new medicines so much! You can understand that...

S. B.: Some people love them too much. Some people carry so many medicines around in their pockets, they have to be healthy just so they're not afraid to take them all! But of course, there are more of the other cases, when a patient needs a certain preparation and it is not in the pharmacy.

A. A.: Is it perhaps the supplier's fault that it's not there?

S. B.: Unfortunately, it does happen that way. A medicine may be available in one pharmacy, but not in the one down the street. And it may be sitting in the central warehouse. When that wrong order was rescinded, we spoke frankly and openly: with all due respect for the pharmacist, he is not the one treating the patient, the physician is. It is not up to the workers in the pharmacy to dictate to the physician which treatment is to be used. The workers in the pharmacy should tell the physician which substances are available, and what the analogs are. You know, our physicians are for some reason timid about turning to reference books. This year I visited polyclinics specifically to see how the physician chooses what he needs from his arsenal fo medicines. Physicians rely only on their memories! In some countries, the physician calmly thumbs through his reference book in front of the patient, without feeling uncomfortable about it, and the patient considers that as the usual way of doing things. Really, who can remember all the prescriptions, all the names. There are thousands of them!

A. R.: There are also incompatible medicines that because of their chemical and pharmacological properties cannot be taken together.

S. B.: That is absolutely right. The reference book should always be close at hand. This is in the interest of the patient. But for some reason, physicians are afraid of losing their authority in front of the patient by using this means of checking oneself.

A. R.: It's a good thing that you are the one to say that. When people read the minister's opinion on this issue, perhaps they will not harshly judge a physician who wants to cheke himself by looking in a reference book. Patients will stop judging and physicians will stop being embarrassed.

S. B.: You had a second question?

A. R.: We recently published an article by the writer, Belov, "Challenge... to a Physician?" It concerned a patient, who, in protecting that which is most dear to him--his health, his life--claims he has the right to go to a "strange" physician whom he trusts more. This opinion elicited such animated and contradictory mail! Patients understandably supported the author unconditionally. Many physicians did too. Probably those were physicians who are not afraid of the right to choose, and are sure that they will not be ignored. There were also some very sharp, even aggressive, letters of protest. Some sent in copies of complaints about the newspaper which they had sent to certain authorities. Their general conclusion: the physician must be beyond criticism. The authors of these letters are sure that physicians must not be criticized, they say that it is not in the best interest of health care. They say that the opposite is required: physicians should be given more authority.

Frankly, I do not quite understand this. I have a lot of friends and acquaintances who are physicians. I have a great deal of respect for them, and I value the exceptional nature of their profession. It is the most noble, and it carries the greatest responsibility. Their mistakes are not

forgiven, and they do not have the right to choose whom they treat. They can be called in the middle of the night. One of my close friends, a physician, was roused at two in the morning. A colleague from the hospital said just one word, "Come". So she got up in the dark. She couldn't get a taxi. She arrived at the hospital in the cab of a street-cleaning machine and gave the driver three rubles--for transporting her.

This kind of physician must be praised. But not all physicians are the same. Why can't we say openly that there are physicians who are not very attentive or very friendly? Do you know that some of them do not even wash their hands when examining a patient? They arrive at the patient's home and do not wash their hands. Should we just say nothing as if we don't notice anything?

S. B.: I'll answer you by saying that physicians are people. There is nothing in human nature that is foreign to them. Among people, and therefore among physicians, there are the good and the bad--there are all different kinds. If a physician does not perform his official durty, then he must be criticized, and not only criticized. I am always glad to hear when the difficulty and selflessness of a physician's work is recognized. The lot of a uchastok physician is hard. He climbs flights of stairs: ten times he runs from one home down to the street, and then on up to another home. And how about the rural doctor! Look at the physicians and nurses who work in resuscitation units. They are under constant stress, always at the limits of their strength. But it would be wrong if behind the backs of these selfless workers, irresponsible, lazy people were hiding, who didn't care about the grief of others. Criticism is necessary if it is done fairly and with a recognition of responsibility.

The question of patients' choosing their own physicians is not a new one. And LITERATURNAYA GAZETA is not the first to discuss it. Even so, it has evoked a strong reaction this time. There is no way we can get away from the principle of territorial medical service. It is in the patients' best interests. Every patient should have a regular physician, who must know the patient's living conditions, home and family situation. It is hard to imagine a physician coming from one end of the city to another to see a patient who has chosen him to be his physician.

A. B.: Well, what if the physician doesn't have to go anywhere--the patient comes to the polyclinic?

S. B.: Fine, another physician he has chosen will examine him. He sees that physician once, twice. But if later, he becomes so ill that he must remain in bed, a physician has to visit him at home. I should say that when a patient starts changing physicians, it does not help him make a quick recovery. If you ruin your hairdo, you can easily fix it, but if your health is ruined by inconsistent treatment or contradictory instructions, the damage is much more difficult to repair.

But the main issue here concerns something else; there are not that many debatable cases. It can happen that there is no communication between

physician and patient if the patient has stopped trusting the physician. In such cases we recommend that the health care agencies refrain from being stubborn. They should not stand on principle. They should provide a different physician. Can the patient just be left without medical help? First of course, the situation must be discussed thoroughly and explained patiently.

A. R.: Thank-you very much, Sergey Petrovich, for the interview and for not evading my pointed questions.

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MEETING OF ADMINISTRATIVE MILITARY MEDICAL EXPERTISE STAFF OF USSR ARMED FORCES

Moscow VOYENNO-MEDITSINSKIY ZHURNAL in Russian No 3, Mar 82 pp 78-79

[Article by A. Ye. Komaristov, Col Med Serv]

[Text] A meeting of heads of military medical commissions of branches of the Armed Forces, military okrugs, groups of forces, fleets and flotillas was held in Moscow, in November 1981. The opening remarks were delivered by F. I. Komarov, Col Gen Med Serv, head of the Central Military Medical Administration of the USSR Ministry of Defense.

A paper dealing with the achievements of military medical expertise and problems of improving it was delivered by V. A. Barannik, Maj Gen Med Serv, head of the Central Military Medical Commission. He stated that, as a result of the persistent joint efforts of public health bodies and institutions, military commissariats and military medical commissions of military okrugs, the main indicators of therapeutic and health-improving work among young men and inductees, medical certification upon registration and induction, screening of candidates for military educational establishments have improved in the last 5 years. Considerable advances have been made in this respect by the military medical commissions of the Carpathian, Leningrad and Kiev military okrugs. Constant upgrading of medical care of servicemen, improvement of the system of dispensary care and quality of therapeutic, diagnostic, preventive and expert work was instrumental in lower rates of dismissal of servicemen and number of cases of disqualifying military specialists for reasons of health. The speaker dwelled in detail on flaws and unsolved problems that are still encountered.

Ye. P. Vshivkov, A. Ye. Komaristov, A. N. Stepanov and A. V. Deyev, Cols Med Serv, shed exhaustive light on the main directions of activity of regular [T/O] military medical expertise bodies. Much interest was displayed in the papers of the heads of military medical commissions of branches of the Armed Forces. The speakers shared with their experience in working on medical screening to man units (chasti) with healthy, physically strong personnel, preservation of servicemen for service in the army and navy, lowering disqualification of military specialists.

The paper delivered by I. V. Sinopal'nikov, Maj Gen Med Serv, summed up the therapeutic-diagnostic and preventive work in the Armed Forces over the last 4 years and discussed the prospects for improving it.

A. N. Zaytsev, Col Med Serv, acquainted the audience with the work of the military medical commission of a district in the area of organizing reception, examination and distribution of young reinforcements in units and subunits [podrazdeleniya] as related to health status and psychophysiological traits.

I. G. Korobushkin, Col Med Serv, delivered a report on military medical expertise of servicemen with sequelae of trauma on the basis of material of the military medical commission of the district. The work of the medical service and military medical expertise bodies dealing with screening of candidates for military educational institutions among servicemen was the topic of G. P. Polishenko, Col Med Serv.

The paper of V. T. Bakshutov, Col Med Serv, dealt with involvement of the okrug military medical commission in tactical and special medical training and problems of organizing military medical expertise in wartime.

In summing up the work of this gathering, F. I. Komarov, Col Gen Med Serv, noted that representatives of military medical expertise are working persistently to meet the requirements of the USSR Ministry of Defense for further improvement of manning the Armed Forces and medical support of army and navy personnel. He raised the tasks of improving screening, placement and training of personnel to be engaged in military medical expertise and pointed to the importance of deeper investigation of ideological-political and professional qualities of expert physicians, of constant advancement of their professional training.

Military medical commissions must continue to improve the style and methods of their work, be more exacting, intensify supervision and checking of performance. There must be further persistent work on improving the quality of training and screening reinforcements for the Armed Forces and candidates for military educational establishments. F. I. Komarov, Col Gen Med Serv, also stressed the importance of improvement of planning and organization of military scientific and scientific research work aimed at better solutions of problems put to the medical service and military medical expertise bodies; he defined the role of the military medical commissions in raising the level of therapeutic-diagnostic and preventive work.

The main efforts of expert physicians must be directed toward rendering practical and methodological assistance to the troop level of the medical service to retain servicemen in active military service. Special attention should be given to comprehensive upgrading of the role and authority of hospital and garrison military medical commissions--the main centers of military medical expertise in the Armed Forces, analysis of outcome of treatment of servicemen as related to introduction to practice of new and highly effective therapeutic and diagnostic methods, as well as improvement of the quality of supervising medical certification of individuals subject to military service.

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SCIENTIFIC AND PRACTICAL CONFERENCE OF PHYSICIANS OF RED BANNER TURKESTAN MILITARY DISTRICT [OKRUG]

Moscow VOYENNO-MEDITSINSKIY ZHURNAL in Russian No 3, Mar 82 pp 79-80

[Article by N. N. Kamenskov, Maj Gen Med Serv, and V. B. Korbut, Maj Med Serv]

[Text] The following participated in a conference that was held in Tashkent: Col Gen Yu. P. Maksimov, commander of the district troops; Lt Gen V. S. Rodin, member of the military council and head of the political administration; Lt Gen A. I. Agudov, deputy commander of district troops in the rear area; representatives of the Central Military Medical Administration, Ministry of Defense; chief medical specialists of the USSR Ministry of Defense; scientists of the Military Medical Academy imeni S. M. Kirov and military medical faculties; administrators of the medical service and chief surgeons of branches of the Armed Forces, military districts and groups of forces; specialists of medical institutions of central subordination.

The participants at this conference discussed questions of medical support of the troops situated in a mountain and desert locality with hot climate. In his opening remarks, Lt Gen A. I. Agudov stressed the importance of studying knowhow in medical support, pointing to the importance of working out practical recommendations aimed at lowering the adverse effects on servicemen of extreme climate and geographic factors, as well as prevention of morbidity among the personnel.

N. N. Kamenskov, Maj Gen Med Serv, head of the district's medical service, devoted his paper to the distinctions of operating the medical service in a mountain and desert region. He noted that local climate and geographic factors have a substantial effect on the nature of troop activities, as a result of which it may be necessary in a number of instances to use methods of organizing medical support that are not provided in the existing regulations ["documents"].

It will be necessary to devote much attention to control of dehydration of the body in the set of therapeutic measures at all stages of medical evacuation. A special role is attributed to proper and prompt implementation of sanitary-hygienic and epidemic-control measures. Ignorance of specific conditions and epidemic situation in the field, as well as delay in implementing the above measures, could lead to a rise in morbidity among personnel. In this paper, there was validation of the principles of deploying and material-technical support of stages of medical evaluation in a mountain and desert region.

Lt Gens Med Serv K. M. Lisitsyn and Ye. V. Gembitskiy, Maj Gens Med Serv S. S. Ilyichenko, B. S. Uvarov, I. I. Deryabin, A. N. Kishkovskiy and B. A. Samotokin discussed important questions of treatment of modern combat trauma and offered valuable suggestions for further improvement of medical support of the troops.

The papers of the chief medical specialists of the district focused mainly on organization of qualified and specialized medical care in subunits [podrazdeleniya], units [chasti] and institutions of the medical service, as well as steps to improve it. Several papers (I. V. Sinopal'nikov, B. A. Samotokin, N. N. Kamenskov, V. I. Kosarev and others) were devoted to questions of medical evacuation.

Representatives from the Military Medical Academy imeni S. M. Kirov, GVKG [Main Military Corps Hospital?] imeni N. N. Burdenko and military medical faculties delivered comprehensive papers. They shared their experience in working in specialized departments of clinics of the Academy, hospital and faculties, and they told about new methods of patient examination and treatment.

There was extensive representation of the work of district physicians. The papers of Col Med Serv I. G. Korobushkin, Lt Cols Med Serv A. I. Askarov, P. N. Zubarev, V. N. Kostenko and others prompted much interest.

In all, there were about 80 people who delivered papers or participated in the discussions. An exhibit of modern therapeutic and diagnostic equipment, rationalization proposals and inventions, military scientific work and other materials reflecting the performance of the medical service of this district over the last 5 years was organized at the conference.

In summing up the work of the conference, Maj Gen Med Serv I. V. Sinopal'nikov pointed to the need to work out a standardized methodology and research program in the field, as well as proper statistical processing of data obtained. He called attention to further improvement of professional training of medical personnel, the increasing role of paramedical and junior medical personnel in implementing modern therapeutic and evacuation measures.

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COMPUTERS USED TO CLASSIFY VIRUSES

Moscow VOPROSY VIRUSOLOGII in Russian No 3, May-Jun 82 (manuscript received 29 Dec 81) pp 8-14

[Article by O. N. Ageyeva, O. G. Andzhaparidze, V. M. Kibardin, G. M. Nazarova and Ye. A. Pleteneva, Moscow Scientific Research Institute of Viral Products, USSR Ministry of Health]

[Text] Expert evaluations and mathematical methods are presently used to create classifications in different branches of science.

The existing classification of viruses was developed by the method of expert rating. This method is based on questioning a large number of specialists and processing of their answers by the International Committee for Virus Taxonomy (ICVT) [1-3].

The construction of any classification is a job of separating a set of objects into disjoint subsets. One of the mathematical methods, namely cluster analysis, makes it possible to separate a set of objects into a certain number of subsets (clusters) in such a way that each object would belong to one and only one of the subsets, so that the objects in a subset would be similar in a certain sense, while objects referable to different subsets would differ from one another.

Thus, the main task of classification is identical to the main task of cluster analysis; for this reason, we have used here the mathematical apparatus of cluster analysis to construct a classification of viruses.

Since there are many modifications of the cluster analysis method, it may be difficult to determine which method is better a priori. The choice of method depends on both the purpose of a study and the type of data. For this reason, the first and independent task was to select the most suitable variant of cluster analysis and test the possibility of using it for our goals.*

*See, for example, [4, 5], concerning the use of cluster analysis for the purpose of constructing classifications in different branches of science.

Material and Methods

In our work, we used an array of viruses, including human, animal, plant and bacterial viruses. Inclusion of viruses in the array was determined primarily by the extent to which they had been studied. The array included only viruses about which at least 75% of all necessary information was known. Analysis of all known viruses enabled us to single out 83 viruses that met this requirement. We considered information about 41 tags [traits] of each virus to be the necessary information. The set of these tags characterizes the main physicochemical and biological properties of virions, as well as some distinctions of viral reproduction in sensitive systems.

Viruses: 1) type 12(A) adenovirus, 2) type 16(B) adenovirus, 3) type 2(C) adenovirus, 4) avian CELO virus, 5) murine adenovirus, 6) type 6(C) adenovirus, 7) type 5(C) adenovirus, 8) simian type 15 (SV 15) adenovirus, 9) simian type 20 (SV 20) adenovirus, 10) simian type 32 (SV 32) adenovirus, 11) simian type 34 (SV 34) adenovirus, 12) simian virus 40 (SV 40), 13) polyoma virus, 14) BK [VK?] virus, 15) human papilloma virus, 16) rabbit papilloma virus, 17) type 1 herpesvirus, 18) equine rhinopneumonitis virus, 19) pseudorabies [Aujeszky's disease] virus, 20) human cytomegalovirus, 21) Epstein-Barr virus, 22) Marek's disease [avian neurolymphomatosis] virus, 23) Lucke's virus, 24) equine herpesvirus, 25) densonucleosis virus, 26) type 1 adeno-associated virus, 27) Kilham virus, 28) Lyu [?]-3 virus, 29) small murine virus, 30) Aleutian mink disease virus, 31) vaccinia virus, 32) feline Calicivirus, 33) swine vesicular exanthema virus, 34) type 1 poliomyelitis virus, 35) type 1 coxsackievirus, 36) bovine enterovirus, 37) Mengo virus, 38) human type 2 rhinovirus, 39) equine type 1 rhinovirus, 40) foot and mouth disease virus, 41) Nodamura virus, 42) Sindbis virus, 43) Semliki Forest virus, 44) Venezuela encephalitis virus, 45) Japanese encephalitis virus, 46) type 2 dengue virus, 47) rubella virus, 48) mouse lactate dehydrogenase virus, 49) Uukuniemi virus, 50) Lumbo virus, 51) LaCrosse virus, 52) vesicular stomatitis virus, 53) rabies virus, 54) baby pike rabies virus, 55) carnivores' plague virus, 56) measles virus, 57) Newcastle disease virus, 58) Sendai virus, 59) type 5 (SV 5) simian paramyxovirus, 60) mumps virus, 61) Pichinde virus, 62) lymphocytic choriomeningitis virus, 63) type A influenza virus, 64) avian plague virus, 65) human coronavirus, 66) avian infectious bronchitis virus, 67) human reovirus, 68) sheep bluetongue virus, 69) cytoplasmic polyhedrosis virus, 70) feline leukemia virus, 71) Rous sarcoma virus, 72) Rauscher's leukemia virus, 73) Moloney leukemia virus, 74) avian myeloblastosis virus, 75) mouse mammary tumor virus, 76) reticuloendotheliosis virus, 77) bacteriophage ϕX174, 78) bacteriophage T4, 79) bacteriophage MS 2, 80) bacteriophage λ, 81) tobacco mosaic virus, 82) potato Y virus, 83) potato X virus.

Tags: type of nucleic acid (NA), single- or double-stranded NA, linear or cyclic NA, presence of poly-A in virion NA, ploidy of virion NA, fragmentation of virion NA, quantity of NA fragments, polarity of NA (+ or - chain), molecular mass of NA, cytosine (C) content of virion NA, adenine (A) content of virion NA, guanine and cytosine (G + C) content of virion NA, coefficient of sedimentation of virion NA, density of virion NA in CsCl (in grams/cm^3), quantity of structural polypeptides in virion, NA content of virion (%), protein content (%) of virion, presence of carbohydrates in virion, presence of lipids in virion, molecular mass of virion (in kilodalton), coefficient of sedimentation of virion (S_w^{20}), density of virion in CsCl (grams/cm^3), dimensions of virion

...presence of membrane and features thereof, type of nucleocapsid symmetry, ... of capsomeres, diameter of nucleocapsid spiral, fragmentation of nucleocapsid, presence of enzymes in virion, site of assembly of capsid, site of assembly of virion, hemagglutinating activity of virion, neuraminidase activity of virion, transforming activity of virus, oncogenicity of virus, sensitivity of virion to ether, type of host, type of NA polymerase (cellular or virionic), type of template for synthesis of messenger NA, presence or absence of precursor proteins.

Some of the tags are quantitative and some are qualitative. The quantitative features are represented by a number that corresponds to magnitude, the qualitative ones are given in the form of parameters which took on a value of +1 and -1 in the presence or absence, respectively, of the tag. Some of the qualitative tags were represented by several parameters. For example, since nucleocapsids of virions have different types of symmetry, the tag showing the type of symmetry was characterized by three parameters: cubic symmetry, spiral symmetry and complex symmetry. In all, there were 64 parameters for each virus.

A matrix (L) of the following appearance was the form, in which these data were submitted:

$$L = \begin{Vmatrix} L_{11} L_{12} \ldots L_{1m} \\ L_{21} L_{22} \ldots L_{2m} \\ L_{n1} L_{n2} \ldots L_{nm} \end{Vmatrix} \quad (n = 83,\ m = 64),$$

where L_{11} is the value of the first parameter of the first virus, L_{12} is the value of the second parameter of the first virus, L_{nm} the value of the mth parameter of the nth virus.

The central concept of cluster analysis is the concept of similarity of objects, if one object (virus) is characterized by a set of tags $\{L_{j1}, L_{j2}, \ldots, L_{jm}\}$, and another by the set of tags $\{L_{i1}, L_{i2}, \ldots, L_{im}\}$, one method of determining their similarity is the distance between them in multidimensional space, the dimensionality of which corresponds to the number of tags used. Distance r_{ij} between the ith and jth viruses is determined by the following formula:*

$$L_{ij} = \sqrt{(L_{i1} - L_{j1})^2 (L_{i2} - L_{j2})^2 + \ldots + (L_{im} - L_{jm})^2}. \qquad (1)$$

One can assess the location of objects in relation to one another in the space of the parameters from the distance. Objects that are similar in properties are characterized by a short distance and those that are not similar, by long distance [6].

*Equation (1) represents the usual Euclidean distance between two points (objects) in m-dimensional space.

Since the above tags characterize different physical and chemical properties of a virion and, consequently, have different units of measurement, they must be reduced to dimensionless values (standardized) in order to be able to work with them simultaneously. Standardization was performed by subtracting the mean and dividing by dispersion. As a result, all of the parameters were commensurate, and most of their values occupied a place in the interval between +1 and -1.

There is no information in the literature concerning some viral features; for this reason, there are blank spaces in the data matrix. Since the known methods of cluster analysis require that these gaps be filled, we selected the following two methods of filling in the missing data. With the first method, we entered the mean value in the place of a missing parameter, which had been obtained by averaging the known values of this parameter in other viruses. With the second method, a random value was used to fill in the gaps. This random value had a normal distribution for quantitative tags and a discrete one for qualitative tags (-1 or +1).

Results and Discussion

We chose one of the agglomerative methods of cluster analysis, which is based on successive combining of objects according to degree of similarity [6].

At the first stage of successive grouping of viruses, 83 groups were formed (in other words, 83 clusters), each of which contained 1 virus. Then the two groups that were located closest to one another and, consequently, resembled each other the most were combined, forming a new group. As a result, the number of groups considered decreases by one and becomes 82. This process can be repeated until all objects are united in one group (cluster). This procedure (algorithm) of combining viruses into groups was effected in the form of a computer program. The results were expressed graphically in the form of dendrograms (trees), the nodes [units] of which corresponded to stages of combination.

Figure 1 illustrates a dendrogram of virus grouping in the case where the gaps in the data were filled with average values of parameters. The boldface lines refer to viral groups corresponding to specific families in the classification system proposed by the ICVT.

As can be seen in Figure 1, at first the viruses with the most similar tags are combined, and up to the eighth stage the combining proceeds on the level of individual viruses; after the eighth stage, there is combining of both different viruses and already formed groups of viruses. At a certain stage of the combining process, clusters are formed which can be identified as families, according to the classification of viruses proposed by the ICVT. Thus, the viruses referred to the Caliciviridae family were combined at the 12th stage, those referable to the Arenaviridae family at the 13th stage, Bunyaviridae family--19th stage and Coronaviridae family--34th stage. Formation of the Picornaviridae family ended at the 39th stage, that of Adenoviridae at the 40th stage, Orthomyxoviridae at the 49th stage, Oncornaviridae subfamily at the 51st stage, Papovaviridae family at the 53d stage, Parvoviridae at the 55th stage, Reoviridae at the 64th stage, Herpesviridae at the 65th and Togaviridae at the 66th stage.

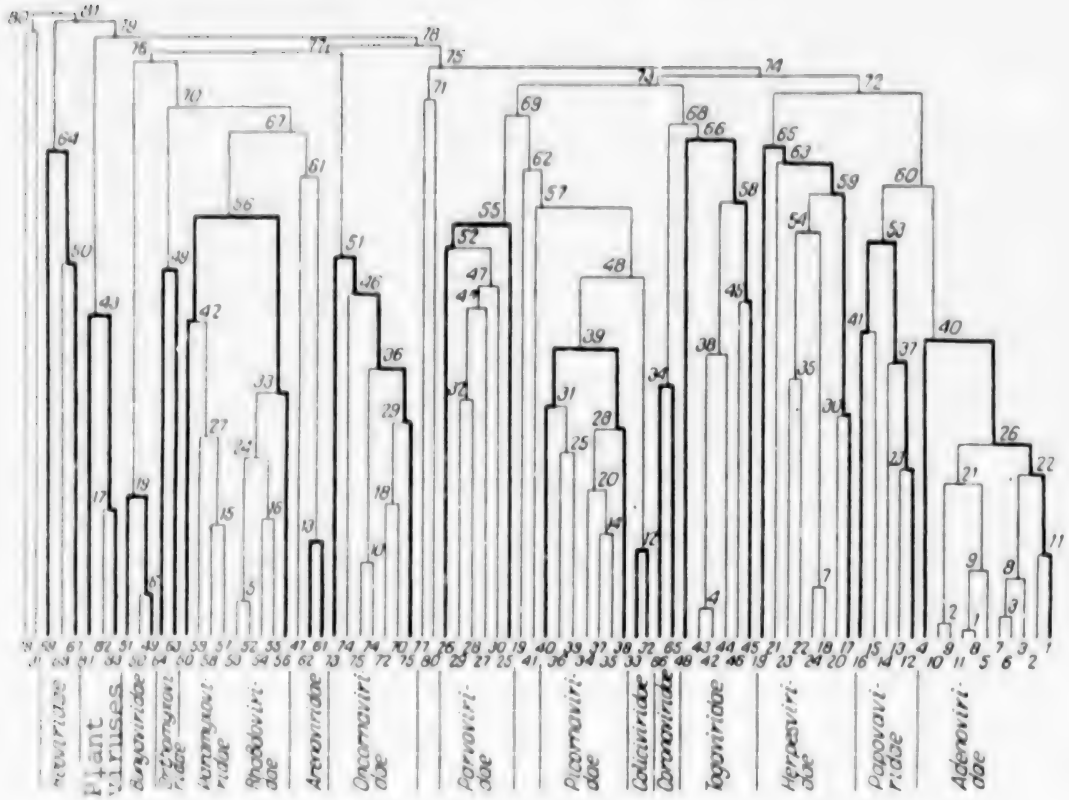

Figure 1. Dendrogram reflecting the process of successive combination of different viruses into groups

Here and in Figures 2 and 3, the numbers at the base of the tree correspond to different viruses. The numbers in tree nodes refer to stage of combination. The groups outlined in boldface correspond to families proposed by the ICTV. Data gaps were filled with average values of parameters.

In addition, there was combination of viruses referable to different families according to the ICVT classification. Thus, at the 16th stage, carnivores' plague virus (paramyxovirus family) was combined with baby pike rabies virus (rhabdovirus family). At the 24th stage, this group was combined with a group that included vesicular stomatitis and rabies viruses, which are two typical representatives of rhabdoviruses. At the 33d stage, this cluster was combined with measles virus. At the 56th stage, this group was combined with a group that contained Sendai, Newcastle's disease, SV5 and mumps viruses (paramyxoviruses).

It should also be noted that there was no formation of a cluster containing all viruses of the Tagaviridae family: there was combination of representatives of the genera Alphavirus, Flavivirus and lactate dehydrogenase virus. Rubella virus was not included in this cluster, being combined with viruses that belong, according to the ICVT classification, to the Arenaviridae family.

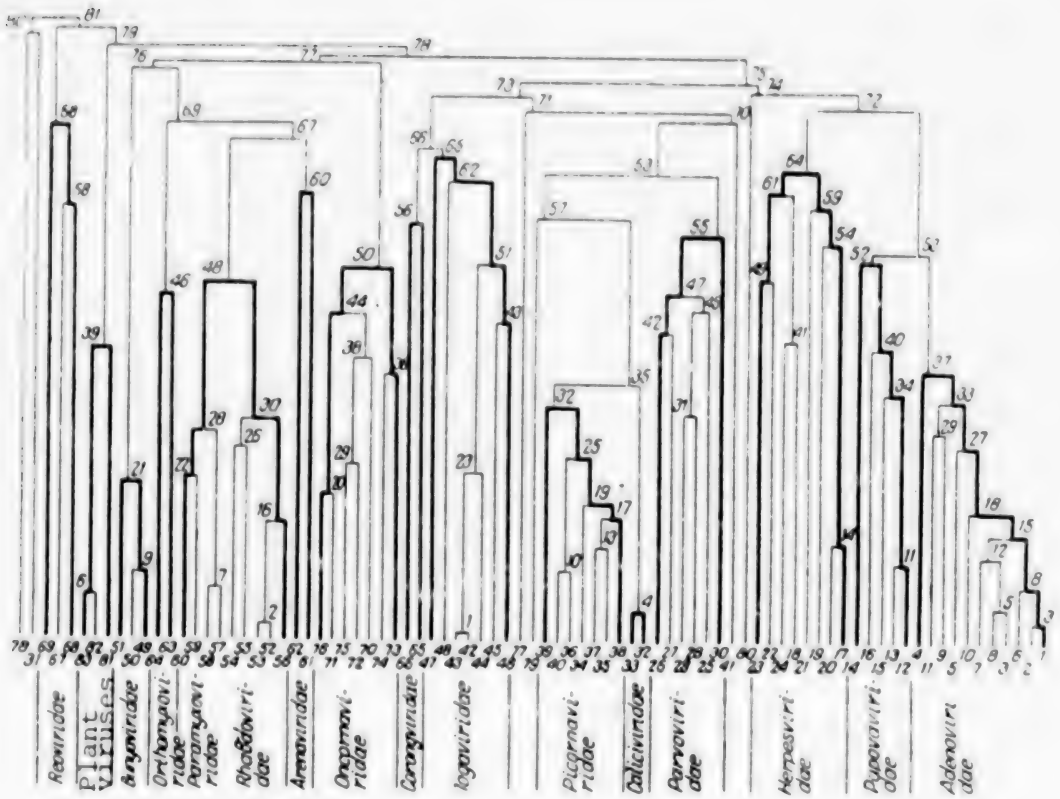

Figure 2. Dendrogram illustrating the process of successive combination of different viruses into groups when missing data are re-replaced with a random figure

The following may be reasons why groups are formed that do not correspond to the ICVT classification: a) presence of information noise that is added at all stages of receiving and processing information or a consequence of absence of information; b) these viruses do indeed differ from the groups in which they were placed by the ICVT.

In order to check the stability of the obtained system for combining viruses and demonstrate its dependence on added information noise, we used two methodological approaches. The first consisted of filling the gaps in the data matrix with a random figure. The second approach consisted of introducing an additional error with dispersion of 0.1 to the values of all parameters used. We analyzed three variants of distribution of viruses in groups, which were obtained by filling the gaps in data by random figures. One of these variants is illustrated in Figure 2.

As shown by analysis of these three variants, the distribution of viruses in groups essenitally conforms to the distribution illustrated in Figure 1. However, we observed instability of the position of measles, carnivores' plague, rubella, lactate dehydrogenase and baby pike rabies viruses. Mixed

clusters were also formed, which included representatives of both paramyxoviruses and rhabdoviruses (see Figure 2, stages 16, 26, 30).

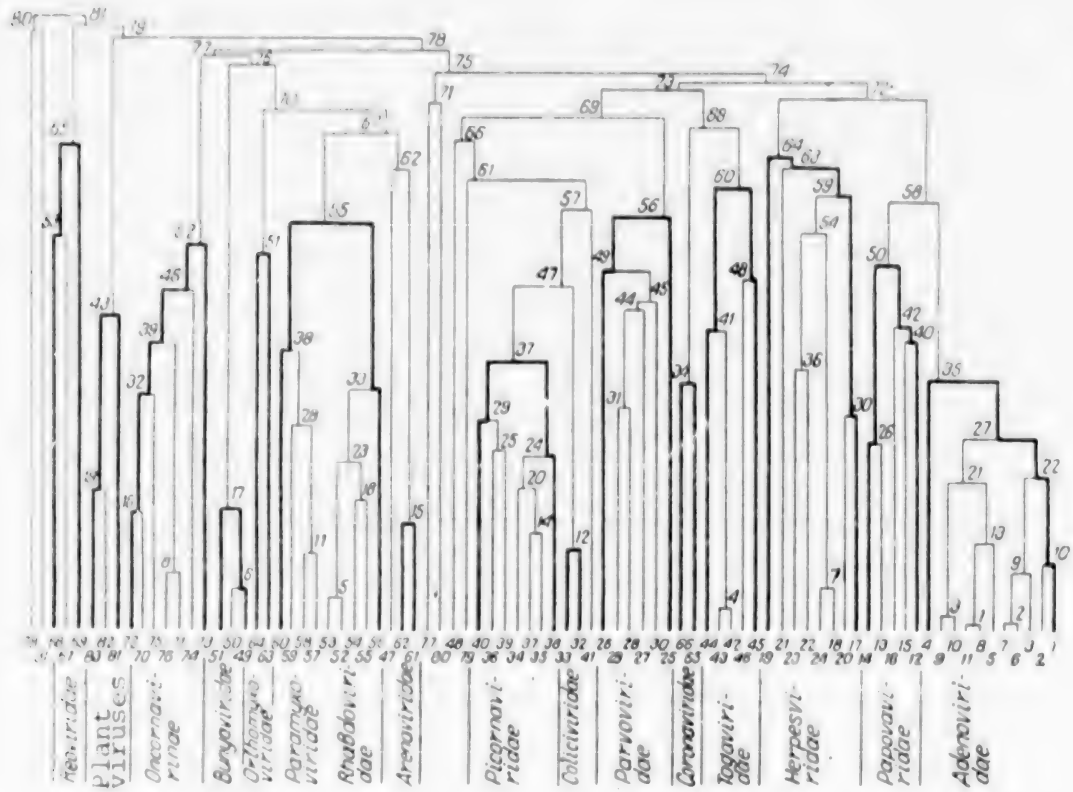

Figure 3. Dendrogram reflecting the process of successive combination of different viruses into groups with use of additional error in values of parameters. Gaps are filled with zeroes

Dendrograms obtained with use of additional error in available data (one of these dendrograms is illustrated in Figure 3) revealed that this change in value of parameters did not have an appreciable influence on distribution of viruses. The only virus whose place changed with the additional error was murine lactate dehydrogenase virus, which was included with viruses of the Picornaviridae family at the late stages of combination.

The results of this series of experiments enabled us to derive the following conclusions: a) the obtained system of distribution of viruses in groups is generally rather stable; this is indicated by the fact that use of additional information interference had no appreciable influence on virus grouping; b) some viruses occupy an indeterminate ("unstable") position in the system of distribution of viruses in groups, as indicated by their particular sensitivity to experimental conditions; this could be due to either a shortage of information about parameters that determine the position of viruses in the system, or their actual difference from the viruses considered.

In order to determine which of these variants could be referred to some "unstable" virus or other, we analyzed the information available about them. This analysis

revealed that there was no information about 17 parameters of lactate dehydrogenase, 15 of rubella, 13 characterizing baby pike rabies virus, 9 parameters of carnivore's rabies virus and 7 parameters of measles virus. At the same time, absence of information about 11 parameters of mumps virus did not prevent it from being constantly placed with viruses of the Paramyxoviridae family. This warrants the assumption that the place of each virus in the system depends not only on the amount of information about it, but the quality of information, i.e., it is possible for different tags to differ in informativeness when used to separate viruses into groups.

Distance between "unstable " viruses and the virus groups closest to them, quantity and existence of parameters constituting 85% of this distance

Virus	Family to which closest virus groups belong	Distance, arbitrary units	Parameters constituting 85% of distance	
			total number	for which there is information
Rubella	Togaviridae	6.54	7	7
	Arenaviridae	6.75	5	5
Lactate dehydrogenase	Togaviridae	6.52	5	5
Measles	Rhabdoviridae	3.89	6	3
	Paramyxoviridae	5.20	5	5
Carnivores' plague	Rhabdoviridae	3.47	5	2
	Paramyxoviridae	5.15	3	1
Baby pike plague	Rhabdoviridae	3.22	6	1

We determined the differences between values of each tag for each of these viruses and averaged value of this tag in the closest virus groups in order to assess the quality of missing information and detect tags that determined substantially the place of "unstable" viruses. We then determined the tags that made the largest contribution to the distance between this virus and a given virus group (let us recall that, according to equation 1, the distance between viruses and virus groups is made up of differences in all tags).

The Table lists the number of parameters for some viruses which yield an 85% distance between a given virus to the closest virus group, and it also shows whether there is information about these parameters.

As can be seen from the listed data, rubella virus is 6.54 arbitrary units away from the Togaviridae family group of viruses and 6.75 arbitrary units away from another virus group that is close, which includes viruses of the Arenoviridae family, i.e., it is at virtually the same distance from both these families. Since all parameters constituting 85% of the distance between rubella virus and both these virus groups are known, it can be assumed that the equal distance of rubella virus from both families is sufficiently objective and rubella virus cannot be put in either of them.

Similarly, according to the data in the table, it can be assumed that murine lactate dehydrogenase virus cannot be referred to the Togaviridae family.

As for measles, carnivore plague and baby pike plague viruses, as can be seen in the table, the absence of information about parameters, difference in which determine the position of these viruses in relation to the closest virus groups, does not allow us to make a choice between the two above-mentioned variants. Only further investigation of the properties of these viruses will make it possible to determine their place in the system of virus classification by means of the method we used.

One of the most important questions in the problem of separating objects into groups is the choice of optimum number of groups. In some cases, one can choose the number of groups a priori, but in most cases the number of groups that would provide optimum division, from the standpoint of the researcher (expert), is determined in the course of dividing the set into classes.

The distance (isolation) of formed groups from one another could be one of the criteria of a "good" breakdown of viruses into groups. [Distance (isolation) of each given virus group refers to the distance from the region occupied by this group to the closest virus that does not belong to this group]. Each stage of separation of the array of viruses into groups is characterized by its own mean distance, which is the mean distance for all groups. It can be assumed that the system of separating viruses into groups that is closest to a natural classification will be characterized by a rather long average distance, or isolation of different virus groups.

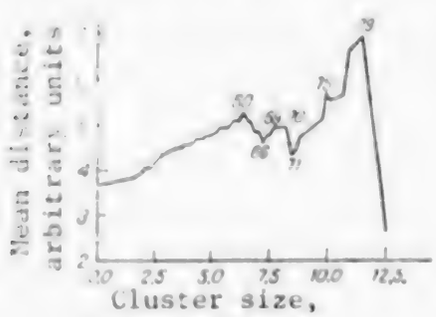

Figure 4.
Averaged distance (isolation) between groups at different grouping stages

The curve illustrated in Figure 4 shows the mean distance of virus groups at different stages of grouping. For the sake of graphic illustration, the change in mean distance of different groups of viruses is considered in relation to the dimensions of the largest group at this stage of grouping. This is quite admissible, since the size of the largest group is directly related to the grouping stage.

As can be seen from this curve, a rather large distance is obtained at the 58th-65th, 69th-70th, 75th and 79th stages of virus grouping. A comparison of groups formed at these stages to the groups in the ICVT classification shows that the 58th-65th stages of clustering correspond to the last stages of formation of groups which, according to the ICVT, have the status of families. The subsequent peak distances apparently correspond to superfamily structures.

Thus, our results are indicative of the suitability of one of the variants of cluster analysis for classification of viruses. The existing array of data enabled us to obtain a specific system of virus classification by means of

this method. Analysis of this system revealed that formation of groups ends at certain stages of division (grouping) of viruses according to degree of similarity of their tags (58th-65th stages); these groups are, on the one hand, quite isolated from one another and, on the other hand, consist of viruses with rather similar properties. A comparison of these virus groups to the classification proposed by ICVT enabled us to establish that they corresponded to different families. Consequently, our analysis of the mutual position of virus groups could serve as the basis for taxonomic ranking and demonstration of hierarchic relations between them.

Analysis of the obtained classification led us to the conclusion that rubella and murine lactate dehydrogenase viruses most probably do not belong to any of the formed families. Caliciviruses should apparently not be singled out as a separate family, since this group of viruses joins with viruses of the Picornaviridae family at rather early stages. Formation of groups corresponding to the Herpesviridae family ends last, which is indicative of the significant heterogeneity of viruses it includes and, perhaps, a need to revise the taxonomic status of some subgroups making up this group. We were unable to determine the place of measles, baby pike and carnivores' rabies viruses by the method we used in view of the lack of information about a number of their tags.

The sufficient objectivity of the results obtained with our approach, the feasibility of comprehensive analysis of the developed system of virus classification in order to solve a number of problems of practical and theoretical virology demonstrate the great potential of this approach, which was used here for the first time in virology.

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CONCENTRATED AND PURIFIED TICK-BORNE ENCEPHALITIS VACCINE: IMMUNOLOGICAL EVALUATION IN EXPERIMENTS ON MICE

Moscow VOPROSY VIRUSOLOGII in Russian No 3, May-Jun 82 (manuscript received 17 Mar 81) pp 60-63

[Article by L. I. Khotlubey, Yu. V. Pervikov, G. L. Krutyanskaya, L. M. Vil'ner, B. F. Semenov and L. B. El'bert, Institute of Poliomyelitis and Viral Encephalites, USSR Academy of Medical Sciences, Moscow]

[Text] We previously published data on primary technological and biological characteristics of a new inactivated, concentrated and purified vaccine against tick-borne encephalitis [1]. We submit here the results of further comparative studies of immunological properties of concentrated and commercial vaccines in one experimental model, white mice, according to parameters of cellular and humoral immunity. These studies were conducted in order to work out approaches for determination of the mechanisms of the vaccinal protective effect on the basis of comparing two products differing significantly in capacity of inducing resistance to infection in mice.

Material and Methods

Viruses: Tick-borne encephalitis virus (TEV), Sof'in strain, and vesicular stomatitis virus, Indiana strain.

Vaccines, immunization of mice: We used two cultural formalin-inactivated vaccines derived from the Sof'in strain of TEV, which reproduced in a primary culture of chick embryos--unconcentrated preparation--intermediate product of commercial vaccine against TEV (PrV); experimental preparation--20-fold concentrated by volume and purified by ultracentrifugation in a saccharose density gradient using a K. Mark II Electronucleonics centrifuge (CV) [1]. The vaccines were given to mice weighing 14-16 g subcutaneously or intraperitoneally in a dosage of 0.25 ml without sorbent.

Protective activity of the vaccines was determined by the maximum dilution that protected 50% of the immunized mice from 100-1000 LD_{50} TEV given intraperitoneally (PD_{50}). The result was assessed 14 days after infection.

Titration of serum antibodies to TEV: Neutralizing antibodies were determined according to 80-100% reduction of plaque-formation by TEV in an SPEV [porcine embryonic renal cells] cell line; the results were expressed as neutralization

index which is the difference between titers in experimental and control (with normal serum) titration, or antibody titer in relation to 30-100 PPU [plaque producing units] of virus. The results were rated to be significant with a neutralization index of ≥ 1.7 $\log_2$ and antibody titer of $\geq 1{:}10$.

We used a micromodification of the Casals method [2] to run the reaction of hemagglutination inhibition, and 1:10 antibody titer was considered significant.

Reaction of suppression of splenocyte migration: We used the capillary variant of the method in [3, 4]. The minimum depression of splenocyte migration at which a positive effect was recorded constituted 15%.

We titrated interferon according to depression of reproduction of vesicular stomatitis virus in a culture of mouse fibroblast tissue [5].

Demonstration of antibodies to ram erythrocytes: We injected a 1% erythrocyte suspension to mice, at the rate of 0.2 mℓ, took blood 10 days later, titrated antibodies according to 50% hemolysis using 5% erythrocyte suspension and fresh guinea pig serum in a 1:20 dilution [6].

Tables 1-3 list the data obtained from one of several similar experiments.

Table 1. Protective efficacy (PD_{50}) of vaccines in experiments on BALB/c mice

Frequency of immunization	Concentrated vaccine				Unconcentrated vaccine			
	Infection with TE, postimmunization day							
	4	7	14	21	4	7	14	21
1	1:16	1:13	1:10	—	1:2	1:2	1:2	—
2	1:40	1:40	1:20	1:20	1:8	1:3	1:2	1:2
3	1:53	1:40	1:45	1:58	1:8	1:20	1:2	1:2

Note: The vaccine was given intraperitoneally at a 28-day interval; 6 mice were used for each dilution to titrate the vaccines.

Results

As can be seen in Table 1, the concentrated vaccine [CV] had a more marked and longer protective effect when given to BALB/c mice. In some cases, 20-29 times less of the concentrated product was required to protect 50% of the animals, as compared to the commercial vaccine (PrV). The protective effect increased with increase in number of inoculations. Analogous results were obtained when we compared the efficacy of the two vaccines on CBA mice and mongrel white mice. A comparative evaluation of indicators of the humoral response after single intraperitoneal immunization of BALB/c mice with PrV failed to demonstrate neutralizing and hemagglutination-inhibiting antibodies, whereas they were demonstrable in 100 and 40% of the animals, respectively, after injection of CV. The frequency of demonstration of serum antibodies and levels thereof, as well as persistence, increased and were reliably higher after the second and third injections in mice given CV.

Table 2. Antibodies in BALB/c mice on 7th, 14th and 21st days after vaccine injections

Frequency of immunization	Type of vaccine	Day blood sample taken	NR		Hemagglut.inhibition	
			%PS	MNI	%PS	GMT
1	CV	7	100	2,5±0,1	40	1:12,6
		14	30	1,9±0,1	0	1:10
	PrV	7	0	0	0	1:10
		14	0	0	0	1:10
2*	CV	7	100	3,5±0,3	80	1:42
		14	100	3,3±0,26	80	1:26
		21	100	3,03±0,24	80	1:26
	PrV	7	100	2,44±0,17	10	1:10,6
		14	50	2,3±0,48	0	1:10
		21	33	2,03—0,23	0	1:10
3*	CV	7	100	4,5±0,25	80	1:49
		14	100	3,5±0,3	80	1:64
	PrV	7	100	2,8±0,12	55	1:13
		14	89	2,0±0,06	50	1:14,9

Note: Asterisks indicate that vaccine was given irtraperitoneally at a 28-day interval. NR--neutralization reaction; %PS--percent positive sera; MNI--mean neutralization index; ±--standard deviation (log_{10}); GMT--geometric mean titer. We tested 10 mice in each experimental group.

Table 3. Formation of hypersensitivity of the delayed type (reaction of depressed migration of splenocytes) in BALB/c mice after injection of TE vaccine

Frequency of immun.	Type of vaccine	Positive responses, % day after successive immunization						
		2	4	7	10	14	21	28
1	CV	0	9	33	71	60	27	0
	PrV	0	17	10	70	45	35	0
2	CV	0	62	33	—	31	20	0
	PrV	0	20	11		17	12	0
3	CV	0	33	37	—	40	12	0
	PrV	0	19	10		27	19	0

Note: The vaccines were given intraperitoneally at an interval of 28 days, and 20 mice were tested in each experimental group.

The advantage of concentrated vaccine was demonstrable when we studied the dynamics of antibody formation in mongrel mice (see Table 2 and Figure). Examination of cellular immunity using the reaction of depression of splenocyte migration revealed that both vaccines elicited a positive reaction in 70% of the animals, starting on the 4th postimmunization day. The impression was gained, which was statistically confirmed when we compared the results according to the criterion of conjugate variant signs, that mice given CV presented a positive reaction more often than those immunized with PrV. In spite of the considerable fluctuation of digital parameters of this test, the conclusion is apparently justified that there was no progressive build-up of intensity and duration of the phenomenon as a function of frequency of vaccination (see Table 3).

Synthesis of virus-neutralizing antibodies in mongrel mice. Dynamics of antibody formation induced by concentrated (1) and unconcentrated (2) vaccines.
X-axis, postvaccination time (days); y-axis, neutralization index (log)

KV and PrV presented equally marked interferonogenicity: serum taken 6-24 h after single immunization with either vaccine was equally active in depressing reproduction of vesicular stomatitis virus. Thus, virus treated with nonimmune mouse serum had an infectivity of $25 \cdot 10^3$ PPU/mℓ, whereas after treatment with immune serum its infectivity titer dropped to $0.5\text{-}3 \cdot 10^3$ PPU/mℓ. The supernatant of cellular debris containing 200 µg protein/mℓ (concentration of cell proteins in PrV does not exceed 200 µg/mℓ) depressed viral reproduction to 1/20-1/25th. The same product, diluted to a protein concentration of 10 µg/mℓ (cell protein concentration of CV does not exceed 10 µg/mℓ) had no inhibitory effect on viral reproduction. This warrants the conclusion that interferonogenicity of PrV was attributable, at least to a significant extent, to admixtures of cell origin. The effect of immunization on capacity to synthesize antibodies to ram erythrocytes was tested in the following manner: BALB/c mice, given vaccine or saline (control) twice at a 4-week interval were given a suspension of ram erythrocytes on the 7th day after the second injection. The geometric mean titers in control groups of animals, as well as those immunized with CV and PrV, constituted 1:17.1, 1:14.9 and 1:12.3, respectively, i.e., they did not differ reliably.

Discussion

It is known that the intensity and significance of different immunity factors are not the same for different viral infections and vaccinal processes [9-13], and they have not been determined with sufficient certainty in the case of immunization against tick-borne encephalitis.

The data we have submitted indicate that both vaccines are immunologically active according to all parameters of specific and nonspecific immune responses that we tested. The concentrated vaccine had a more marked and longer protective effect, it had a greater capacity to induce antiviral antibodies, as

demonstrated in the hemagglutination inhibition reaction and neutralization reaction, as well as cellular immunity according to the results of the test for inhibition of splenocyte migration. At the same time, CV and PrV had about the same interferonogenicity and did not affect overall immunological reactivity of mice, which was determined in two tests--capacity to synthesize antibodies to ram erythrocytes and capacity of peritoneal macrophages to submit E. coli to phagocytosis. A comparison of the results of assessing the cellular and humoral responses to the dynamics of formation of protective activity after immunization of CV and PrV suggests that mouse resistance to TEV infection is related, to an appreciable extent, to antibody production. The differences in levels of protective activity of the two vaccines, degree of build-up and duration of protective effect with each successive immunization show a much better correlation to titers of antiviral serum antibodies than hypersensitivity of the delayed type or other tests used. For this reason, we consider it necessary to pursue a more detailed study of functions of T and B systems of immunocompetent cells, in particular, by using the method of adoptive transfer of different fractions thereof. As of now, we have data to the effect that the protection is provided by the cortisone-sensitive fraction of splenocytes. Our hypothesis that antibody production plays the predominant role in assuring protection of immunized mice against TEV infection requires, of course, more direct proof.

We wish to express our appreciation to G. Kh. Kodkind for statistical processing of the data of this study.

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HETEROGENEITY OF VIRUS-SPECIFIC FLAVIVIRUS PROTEINS

Moscow VOPROSY VIRUSOLOGII in Russian No 3, May-Jun 82 (manuscript received 8 Oct 81) pp 64-67

[Article by A. I. Zhankov, T. I. Dzhivanyan and V. A. Lashkevich, Institute of Poliomyelitis and Viral Encephalites, USSR Academy of Medical Sciences, Moscow]

[Text] Nine virus-specific proteins are synthesized in cells infected with flaviviruses: P98 (NV5), P71 (NV4), P51 (E), p44 (NV3), p32 (NVX), P21 (NV2½), P19 (NV2), P14 (C) and P10 (NV1) [1], two of which, P51 (E) and P14 (C), are intracellular forms of structural proteins E and C, previously known as V3 and V2, respectively [2, 3]. Virus-specific proteins of flaviviruses have been studied mainly on models of viruses transmitted by mosquitoes [2-7], and there are only a few works dealing with viral proteins synthesized in cells infected with flaviviruses transmitted by ticks [8-11]. No comparative studies have been made within the framework of the same experiment of virus-specific proteins of viruses transmitted by ticks and viruses transmitted by mosquitoes. We undertook here a comparative study of intracellular proteins of three viruses referable to the tick-borne encephalitis group (TEG) and West Nile virus, which is included in one of the serological subgroups of flaviviruses transmitted by mosquitoes.

Material and Methods

Viruses and cell culture: We used TEG viruses (Sof'in strain of tick-borne encephalitis virus--TEV, Bayers strain of Powassan virus, strain TP-21 of Langat virus) [10] and West Nile virus [12]. All of the viruses were incubated in monolayer cultures of transferable pig embryo renal cells (PER), which were cultivated by a previously described method [13].

Recovery of labeled virus-specific proteins: The blended monolayers of PER cells were infected with multiplicity of about 1 PPU [plaque-production unit]/cell; the viruses were cultivated in medium 199 with Earle's solution and 2% normal calf serum. Infected cells were labeled with a mixture of ^{14}C-labeled amino acids at different postinfection times. Three hours before the start of tagging, the viral cultivation medium was replaced with Earle's solution with 2% normal calf serum and actinomycin D (5 μg/mℓ) and cells continued to be incubated at 37°C; then the monolayer was eluted 3 times in Earle's solution and submitted to lysis in a buffer for electrophoresis of the following composition: 0.0625 M tris-HCl, pH 6.8; 2% SDS, 5% 2-mercaptoethanol, 8 M urea,

10% glycerin, 5 mM EDTA and 0.001% bromphenol blue. Uninfected control cells were treated in the same manner.

Electrophoresis in polyacrylamide gel (PAAG) and fluorography: Electrophoresis was conducted on PAAG plates in an intermittent buffer system [14]. Gels were prepared for fluorography [15] and exposed for different periods of time using RM-1 film.

Reagents: Actinomycin D, Serva Co. reagents for electrophoresis and UVVVR Firm (CSSR) ^{14}C-labeled protein hydrolysate.

Results

The dynamics of appearance and accumulation of intracellular virus-specific proteins were studied at different stages of infection of PER cells (Figure 1).

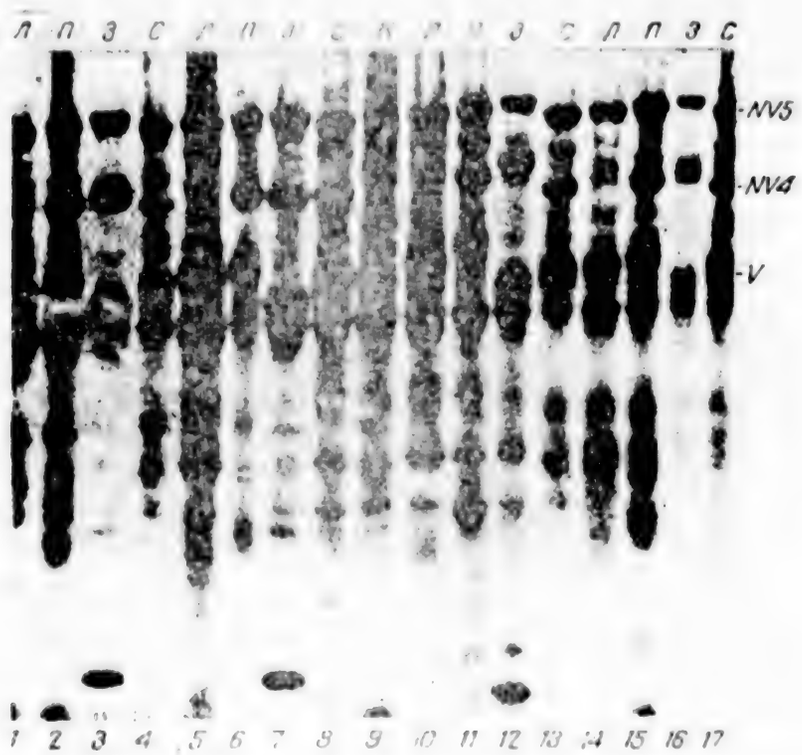

Figure 1. Fluorogram of proteins from infected and noninfected PER cells after electrophoresis in 10% PAAG

Here and in Figure 2:

Л) proteins recovered in electrophoretic analysis of PER cells infected with Langat virus
П) infected with Powassan virus
З) with West Nile virus
С) with Sof'in
К) noninfected control cells
1-4) proteins formed in cells labeled with ^{14}C between 24th and 30th h after infection
5-8) cells labeled between 30th and 36th h
10-13) cells labeled between 36th and 42d h
14-17) cells labeled between 42d and 48th h

Virus-specific NV5 protein: This protein was demonstrable in all viruses examined when the infected culture was labeled between the 12th and 18th h, 18th and 24th h, but to minimal extent (data not submitted). At the start of the 2d day of infection (columns 1-4 in Figure 1), NV5 accumulated and was distinctly identifiable to the end of the 2d day. Electrophoretic mobility of this polypeptide was the same at all tested times in each individual virus, but there was heterogeneity of NV5 in different viruses; there was very similar, if not identical, mobility of these proteins in TEV and Langat virus; it was relatively lower in Powassan and West Nile virus, but the decline was more marked in West Nile Virus (see Figure 1).

Virus-specific NV4 protein: Like NV5, NV4 protein appeared at the end of the 1st postinfection day (data not submitted). It was well-marked at all tested phases in Powassan, West Nile and TEV viruses. In contrast, protein NV4 was represented by a mild band in Langat virus, showing mobility similar to that of the same protein in TEV (see Figure 1, Л). NV4 protein of TEV presented the same mobility at all times. Similar characteristics were demonstrated in the analogous protein of Powassan virus at the start of the 2d postinfection day (see Figure 1, *2* and *6*), but by the end of the 2d day there was a tendency toward decreased mobility (see Figure 1, *1*, *2*, *6* and *11*, *15*). NV4 of West Nile virus was first demonstrable as a band with somewhat less mobility than the same proteins in TEV and Powassan virus (see Figure 1, *1*, *3* and *7*), after which it was identified as having even less mobility than in Powassan virus.

Zone between virus-specific proteins NV5 and NV4: At the start of the 2d postinfection day, an additional protein, apparently virus-specific, appeared in the indicated region in TEG viruses, which was not demonstrable in the noninfected PER control cells (see Figure 1, *1*, *9*) or at the early stages of infection (data not submitted). A similar protein was demonstrable in West Nile virus in the form of a faint band, which was entirely absent at the early stages of infection and some of the late stages. Mobility of this protein varied significantly, even in the same virus, at different times, which is indicative of instability of its characteristics.

Intracellular form of virus-specific structural protein V3 (E): This protein can be readily differentiated in infected cells starting at the very earliest stages of infection (data not submitted) and up to the latest ones (see Figure 1). The mobility of this protein was very similar in Langat virus and TEV; it was somewhat higher and lower in West Nile and Powassan viruses, respectively.

All of the above data were reproduced in several repeated experiments.

We studied virus-specific proteins not only in 10% PAAG, but 12.5% and 7.5% PAAG. Examination of polypeptides in 12.5% gel virtually failed to demonstrate heterogeneity among the large virus-specific proteins, with the exception of insignificant differences in mobility of the intracellular form of structural protein V3 (data not submitted). On the other hand, electrophoresis in 7.5% gel revealed that NV5, NV4 and V3 proteins of different viruses differed significantly in mobility and presented similar, but more marked differences from one another than analogous findings with electrophoresis in 10% gel (Figure 2).

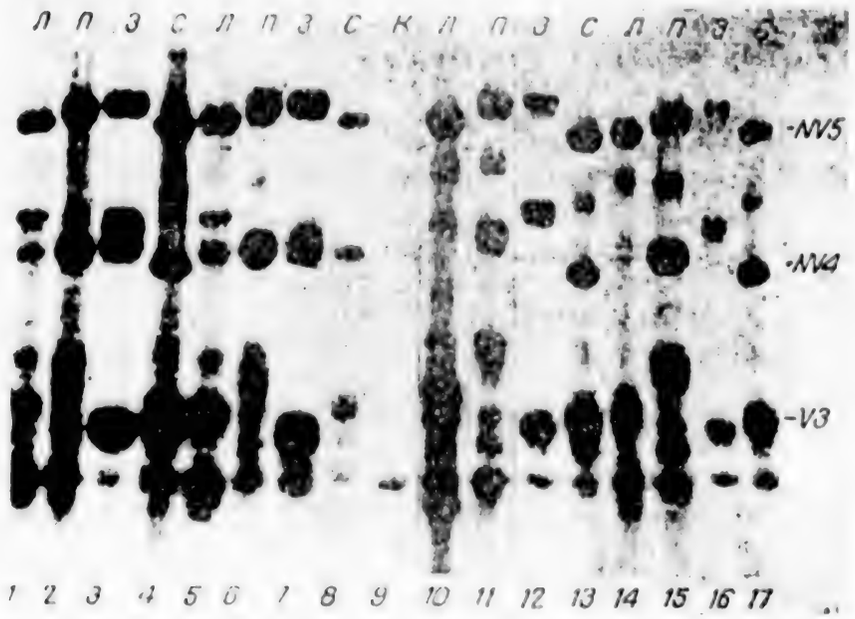

Figure 2. Fluorogram of proteins from infected and noninfected PER cells after analysis in 7.5% PAAG

Discussion

We have submitted here some results that are indicative of differences in electrophoretic mobility of large intracellular virus-specific proteins of different flaviviruses. The electrophoretic conditions we used enabled us to compare the mobility of high-molecular nonstructural proteins, NV5 and NV4, and the intracellular form of structural protein, V3. Previously, in a study of 12 flaviviruses referable to 5 serological subgroups it was shown that polypeptides NV5 and NV4 presented very similar mobility, not only in viruses belonging to the same serological subgroup, but viruses from different subgroups [7]. At the same time, it was reported that intracellular protein V3 and low-molecular nonstructural protein NV2 differ in mobility in the viruses examined. Our data are consistent with the cited results with reference to V3 protein, but differ for NV5 and NV4 proteins. The demonstrated differences can be explained in the following manner. In [7], a study was made of viruses transmitted by mosquitoes, whereas here we studied viruses transmitted by ticks and only one virus (West Nile) transmitted by mosquitoes. PER cells were used for viral reproduction here, whereas Vero and LLC-MK2 cells were used in [7]. In conducting SDS electrophoresis, we used an intermittent tris-HCl system, whereas in [7] an SDS-phosphate continuous buffer system, pH 7.2. was used. In addition, we used proteins in a solution with a high urea content.

It was demonstrated [9] that PER cells infected with TEV synthesize protein with molecular mass of 79,000 dalton, which was designated as NV4½. Later on [11], using peptide maps of tryptic lysates of NV4 and NV4½ proteins, it was shown that these proteins have the same amino acid sequences and that the NV4½ polypeptide is a precursor protein. In our study, we demonstrated a polypeptide

in the zone between NV5 and NV4 proteins at the start of the 2d postinfection day, which did not appear in noninfected control cells. Late appearance of NV4$^1_2$ protein can apparently be attributed to processing of this polypeptide and accumulation of NV4 protein. The differences in electrophoretic mobility of NV5, NV4 proteins and the intracellular form of V3 protein apparently reflect differences in the primary structure of each of these proteins in the viruses studied.

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AFLATOXINS AND THEIR PRODUCERS AS AN IMPORTANT ASPECT OF THE MYCOTOXIN PROBLEM

Alma-Ata ZDRAVOOKHRANENIYE KAZAKHSTANA in Russian No 6, Jun 82 pp 27-34

[Article by P. S. Nikov and S. K. Imankulova, Kazakh Branch of the Institute of Nutrition, USSR Academy of Medical Sciences]

[Text] According to data in the worldwide literature, a distinct tendency toward increased contamination of products of both plant and animal origin by xenobiotics--foreign impurities with properties that are deleterious to man and animals--is observed in connection with the scientific and technological revolution, as well as intensive growth of industry and agriculture.

Xenobiotics constitute a large and extremely diverse group of substances. We can mention among them heavy metals, which are sometimes the waste from industry, pesticides, certain mineral fertilizers, growth stimulators, antibiotics, hormones, nitrosamines, polycyclic hydrocarbons and others. Foreign substances can also appear in foods in the course of technological processing thereof with use of both traditional and new procedures for preserving and cooking food, as well as because of insufficiently validated use of some food additives.

Mycotoxins, which are toxic substances produced by microscopic fungi, form an extensive group of xenobiotics that contaminate foodstuffs.

At the present time, more than 300 such toxic substances have been isolated from foods and identified. They are referable to different classes of chemical compounds and have a wide diversity of effects on the organism. Human and animal diseases caused by them are united by the general concept of "mycotoxicosis."

The toxic metabolites of fungi can attack all organs and tissues. However, many of them are characterized by the fact that they attack predominantly specific organs and systems. We know of mycotoxins that attack mainly the hemopoietic, digestive, nervous, vascular, urinary and other systems.

Various biological effects of mycotoxins have been described in the literature, including general toxic, embryotoxic, teratogenic, oncogenic, mutagenic and allergic effects.

Because of the extreme hazard to human health, mycotoxins have become a worldwide problem, the subject of close attention on the part of special institutions of the World Health Organization. Moreover, since they are the cause of

livestock diseases and death, they inflict serious economic losses. At the present time, 1000 of the 100,000 known species of microscopic fungi are involved in spoiling foods and feed. The worldwide loss due to uncontrolled development of mold fungi on agricultural products and industrial raw material is in excess of 30 billion rubles per year.

The global nature of the problem is attributable to the fact that microscopic fungi, including their toxic representatives, are distributed everywhere. The latter is due to their high adaptive properties, which enable them to inhabit all of the ecological and geographic regions of the world.

Aflatoxins--Classification, Main Mechanisms and Conditions of Biosynthesis

Discovery and identification of the chemical structure and biological properties of aflatoxins produced by A. flavus, A. parasiticus and, according to some data, other fungus species constituted a perceptible event in development of modern mycotoxicology.

Aflatoxins were discovered in 1960 as a result of investigation of mass scale outbreaks of an unknown disease among farm animals and fowl in England, Kenya and Uganda. Necropsies of dead animals revealed acute necrosis of the liver with proliferation of bile ducts. Several researchers demonstrated the link between disease and death of animals, on the one hand, and feeding them meal ground from Brazil nuts contaminated with A. flavus fungus. The toxicity of this meal to domestic and laboratory animals was proven, and the toxic element--aflatoxin--was isolated; it derived its name from the first letters of the name of the fungus that produces it--Aspergillus flavus toxins.

Thin-layer chromatography of aflatoxins isolated from peanut meal resulted in separation thereof into four components: B_1, B_2, G_1 and G_2. They differ in fluorescence under ultraviolet light. The first two are characterized by light blue fluorescence and the last two, green. It has been established that aflatoxins are dehydrofuran derivatives. In addition to the main representatives we have mentioned, this group also includes several toxins with similar chemistry and biological properties: M_1, M_2, B_{2a}, G_{2a}, M, P_1 and Q_1. Compounds such as aflatoxicol, aspertoxin, parasiticol, sterigmatocystin and its derivatives--O-methylsterigmatocystin, 5-methoxysterigmatocystin and 5-hydroxysterigmatocystin--are also referable to aflatoxins.

Chemical methods of demonstration and identification of aflatoxins are based on their intrinsic fluorescence under ultraviolet light, differences in mobility with thin-layer chromatography and specificity of absorption spectrum and fluorescence.

These toxins are notable for considerable resistance to physical and chemical factors: high temperature, ultraviolet and α-rays, changes in medium pH and alkalinity. However, aqueous solutions are readily destroyed in the light. Their high resistance makes it difficult to work out measures to decontaminate them in foods.

Among the factors that affect synthesis of aflatoxin, methion is made of metal ions, particularly Zn^{2+}, which plays a key role in biosynthesis of secondary

metabolites of fungi. Fe^{2+}, Cu^{2+}, Mo^{2+}, Mg^{2+}, thiamin, ethanol and a number of amino acids are known to be stimulators of aflatoxin synthesis, while V^{2+}, Ba^{2+}, carboxylic acids, chelate-forming agents, ethionine and dichlorophos are known as inhibitors. In addition, there are several reports in the literature about the effects on aflatoxin production of such factors as medium pH, salts, alkali, gases, pesticides, etc.

Ambient temperature and humidity, nature of nutrient substrate and degree of its aeration are the principal conditions that influence the toxin-producing capacity of fungi. We can mention the influence of the geographic factor, which is made up of different sets of the above conditions that determine, to some extent or other, manifestation of fungal toxinogenicity. Apparently it is for this reason that the incidence of aflatoxin-producing strains is different in different geographic zones. Thus, the quantity of A. flavus strains that produce aflatoxins isolated from foods is in the range of 6% in India, 70% in Israel and 90-100% in the United States (L. S. L'vova et al.).

Biological Properties and Hygienic Aspects of Contamination of Foods by Aflatoxins and Their Producers

Among other issues related to aflatoxins, researchers devote special attention to their biological properties. There are many studies dealing with identification of the latter.

Analysis of the literature shows that aflatoxins are extremely potent, mainly hepatotoxic and hepatocarcinogenic agents.

In spite of the similarity of chemistry, they differ in severity of toxic and carcinogenic effects. Aflatoxin B_1 demonstrates the highest activity; it is followed by B_2, M_1 (G_1) and M_2 (G_2). The toxicity of aspertoxin, B_2, G_{2a} and sterigmatocystin is 25, 60, 100 and 250 times less, respectively, than that of aflatoxin B_1.

Analysis of the literature shows that aflatoxins are universal toxic agents that attack animals on the most varied steps of evolutionary development. It was found that primates are also sensitive to their deleterious effects.

The carcinogenic dosage of aflatoxins when ingested regularly with food fluctuates over a wide range, depending on the species of animal. Thus, the dosage is 0.1 μg for the rainbow trout and 1-15 μg/kg feed for albino rats. Primates develop cancer of the liver with daily intake of about 0.4 mg aflatoxin B_1 for 6 years.

The severity of the biological effect of aflatoxin depends not only on the animal species, but age and sex, and, according to some data, on diet (A. A. Pokrovskiy et al.).

On the basis of the results of several studies (R. C. Shank; P. Lafont, I. Lafont), it should also be considered proven that humans may also be stricken. Some studies established a distinct correlation between incidence of primary cancer of the liver among the inhabitants of some parts of the world, presence and degree of contamination of foods by aflatoxins. These and a number of other

observations, as well as the fact that most animal species, including primates, are sensitive to aflatoxins warrants the belief that man is no exception as the possible victim of neoplastic lesion induced by these agents.

Among other, less studied biological effects of aflatoxins, we should mention teratogenic and mutagenic effects, which have been demonstrated on a limited number of biological objects. Isolated studies have shown that aflatoxins can have an adverse effect on immunoreactivity.

As we have already noted, two species of fungi referable to the A. flavus-oryzae section are considered to be the main producers of aflatoxins--A. flavus and A. parasiticus. The question of distribution of these fungi and their toxic metabolites occupies a significant place in the world literature. These fungi strike virtually all known foods, being distributed in all ecological and geographic regions of the world, with perhaps the exception of only regions in the extreme north and south. Soil is the natural reservoir of A. flavus and A. parasiticus as, incidentally, it is for other species of microscopic fungi. Water, air and insects play a large part in the spread of the spores of these fungi in the environment.

In the summary of I. Bainton and B. D. Jones, they review the results of studying aflatoxin content of food and feed in the world for the period from 1970 to 1976. According to their data, in Africa, 1594 out of 2965 samples of oil cakes or meal from oil-bearing plant seeds and 152 out of 13,089 samples of edible nuts contained aflatoxins. In North America, they were found in 1 out of 70 samples of feed, 57 out of 978 samples of nuts and 184 out of 1295 samples of oil-bearing plants; in South America, in 258 out of 461 samples of oil-bearing plants and 12 out of 15 tested samples of plant products. According to the same authors, aflatoxins were found in Asia in 24,840 out of 25, 569 tested samples of oil-bearing products in a concentration of 100-1000 μg/kg; in Europe, 90.6% of the examined samples of peanut meal and 11.3% of feed samples were contaminated by these toxins.

There are studies dealing with distribution of aflatoxins and producers thereof in other foodstuffs. Toxigenic strains of A. flavus were found in grain and leguminous products in different regions of Baghdad: 19.4% of wheat samples, 29.4% rye, 20% spaghetti, 33% green peas, 22% sesame, 8.3% Turkish nut [filbert?] and in other food substrates.

H. Spicher reported contamination of flour by 40 species of fungi, incuding A. flavus, which resulted in considerable contamination of bakery goods. Under such conditions, the author detected aflatoxins in various types of bread, more often wheat and rye bread.

According to the data of H. Peterson et al., contamination of oats with A. flavus spores reached $1.1 \cdot 10^5$/g grain in Sweden, and this was associated with significant contamination of the product with aflatoxins (2.6 mg/kg).

In France, aflatoxins were found in 57% of tested wheat samples and 26% of corn samples. In the United States, the figures were 0.4 and 2-3%, respectively.

In Nigeria, aflatoxin B_1 was found in rice, millet, corn and maize, in concentrations of 182.5, 262, 280 and 150.8 μg/kg, respectivley. In protein-rich foods in different regions of this country, maximum amounts of aflatoxin B_1 were

found in samples of dry fish (650 μg/kg) and kidney beans (125 μg/kg). At the same time, negligible amounts of aflatoxins were demonstrable in foods with high carbohydrate content.

In Yugoslavia, 820 samples of foodstuffs were examined over a period of several years. Aflatoxin was detected in 7.5% of the samples, most often in confectionary goods made of peanuts. Aflatoxin was found in concentrations of 1 to 25 μg/kg in peanuts, coffee, barley and wheat imported in this country.

Beneficial conditions for development of A. flavus and formation of aflatoxin in foodstuffs were found in Burgasskiy District of Bulgaria, especially in the southern coastal region.

Aflatoxin-producing strains of A. flavus have also been isolated from foods in some parts of India and Japan.

Several works report the distribution of aflatoxin-producers and aflatoxins in foodstuffs of our country.

According to the data of V. P. Bogoroditskaya, 58.3% of the samples of wheat grain were contaminated with A. flavus, 5.1% of whose strains are producers of aflatoxins. Contamination of cereal grain by A. flavus is also reported in the work of L. S. L'vova et al. According to the results of their studies, 12% of the strains isolated from wheat and corn kernels have aflatoxinogenic properties. Among the cultures of A. Flavus isolated from 307 samples of wheat grain from the harvest of 1972-1973 in different regions of the USSR, 6.8% of the strains had the capacity to produce aflatoxin.

The studies of the staff of the Laboratory of Sanitary and Alimentary Mycology, Kazakh Branch of the Institute of Nutrition, USSR Academy of Medical Sciences, revealed considerable contamination by A. flavus of foodstuffs in different geographic regions of Kazakhstan (A. S. Bukharbayeva et al.; L. M. Fadeyeva et al.; P. S. Nikov et al.). Cases of contamination of foods by aflatoxins have also been reported. Aflatoxin-producers and their toxic metabolites have also been found in imported products used to process confectioner's goods. However, systematic studies of this matter have essentially only begun in our country and, of course, must be continued.

Detection of aflatoxins in products of animal origin--milk, eggs, meat, fish--merits attention. Thus, aflatoxins in a concentration of 1-250 μg/ℓ have been discovered in Iran in 50% of the samples of milk coming from small farms, which supply 85% of the population. A. flavus has also been detected in powdered milk, and for this reason the question is being raised of a need to revise the microbiological standards for this product, as well as change in packaging technology.

Aflatoxin was demonstrated in the liver in amounts of 0.3% and in muscle tissue in amounts of 0.1% of ingested dose among broilers given feed containing this agent.

When Japanese quail and chickens were fed a diet containing aflatoxin in doses of 40 to 10,000 μg/kg, it was demonstrable in their eggs. With increase in

aflatoxin content, the amount thereof in eggs increased. The quail ceased to lay eggs with higher concentrations. A. flavus was detected in "kurta" [?] and dry meat prepared by the traditional method at home in some parts of Kazakhstan.

The wide distribution of aflatoxin-producers and aflatoxins, and the related potential hazard to human health made it necessary to set standards for the latter in foodstuffs. It must be noted that the maximum permissible concentration in foodstuffs fluctuates over a rather wide range, from 5 to 30 μg/kg, in different countries.

Morphophysiological and Hygienic Aspects of Aflatoxinogenicity of Fungi

The successful solution of a range of medical and, in particular, hygenic problems related to aflatoxigenic fungi depends on answering questions pertaining to their physiology and biochemistry, characteristics under different geological conditions, effect on them of biocenotic factors, distinctions of habitat substrate and associations of microorganisms.

For this reason, it is deemed important to define the fungi that are capable of synthesizing aflatoxins. According to some data, in addition to the fungus species mentioned, aflatoxin production is inherent in A. effusus, A. oryzae, A. terricola, A. amstelodami, A. tamarii, A. terreus, Fusarium equiseti, F. moniliforme, F. semitectum, Penicillium puberulum, P. nigricans and fungi of the genus Penicillium that have not been defined as to species. It is believed that better validation is needed to classify these fungi as aflatoxin producers, using modern methods of identification of aflatoxins, which rule out possible artefacts in such identification.

As we have already mentioned, not all strains of A. flavus produce aflatoxins. With this in mind, H. Murakami et al. recommended that all aflatoxin-producing strains be labeled A. parasiticus. However, this suggestion did not gain broad support, and the name of A. flavus continues to be used to refer to both aflatoxin-producing and nonproducing strains of this species.

The indicators of incidence of aflatoxinogenicity in A. flavus strains fluctuate significantly in different literature sources. We cannot rule out the possibility that this property belongs to the species as a whole, while the incidence of its individual expression is apparently determined by the different living conditions of the fungus. In view of this, the opinion held by F. W. Parrish et al. to the effect that many strains of A. flavus are incapable of producing aflatoxin appears debatable and requires further investigation.

With reference to the need for comprehensive studies of various aspects related to aflatoxin-producers, we must mention the inadequate development of such questions as genetic and regulatory mechanisms that determine toxigenicity or nontoxigenicity of strains and the species as a whole.

We should also note that there have been few studies of ontogenesis, morphogenesis and metabolism of A. flavus strains that synthesize aflatoxins. Some authors have tried to study these aspects in order to gain deeper ideas about

the physiology of this fungus and discovery of possible morphological and biochemical traits for differentiation between strains that do and do not produce aflatoxin. The search for differential criteria is related to the fact that, as indicated above, not all strains of the A. flavus fungus synthesize aflatoxins under the same conditions of their natural habitat and artificial cultivation. Establishment of such criteria would aid in better and more economical selection of some strains for research and practical purposes, including industrial.

It should be stressed that the results of such searches are diverse. Thus, C. W. Hesseltine et al. believe that, in principle, it will be possible in the future to identify aflatoxinogenic strains according to morphological traits. Somewhat earlier, H. Murakami et al. discovered a correlation between certain morphological and physiological characteristics of A. flavus strains, on the one hand, and their aflatoxin-producing capacity, on the other. In the opinion of these authors, a certain statistical analysis of several morphophysiological features (shape and dimensions of conidia and conidiophores, pigment production, lysis of H_2O_2 and others) could be used as a preliminary method for screening aflatoxin-producer cultures.

M. M. Pidoplichko et al. have described the morphological characteristics of 130 strains of A. flavus isolated from various substrates in many geographic regions of the world. They observed that the strains synthesizing substances of the aflatoxin type are referable predominantly to the group of lightly colored representatives of this species.

Some isolated studies were concerned with the question of correlation between production of specific types of aflatoxins and presence in the fungus of such morphological traits as sclerotium formation and others.

It should be stressed that such studies, which demonstrate a correlation between function and moprhological properties, have been also made of other fungus species. For example, it was reported that there is a correlation between morphological properties of Penicillium chrysogenum and its capacity to produce penicillin.

In addition to the ones mentioned, a search was undertaken for a relationship between synthesis of aflatoxin and a number of physiological and biochemical properties of A. flavus. Thus, C. W. Hesseltine et al., expounded the assumption, on the basis of their own data and the literature, that when A. flavus strains synthesize large amounts of kojic acid they do not produce aflatoxin, and vice versa. However, these authors believe that this correlation requires further investigation.

To refer to the history of research in the relationship between toxigenicity of fungi and their cytochemical characteristics, we could mention several studies. Among them, we should single out the publication of Yu. I. Rubinshteyn, which indicates that there is a link between nature of fluorescence of Fusarium sporotrichiella hyphae stained with acridine orange and presence, as well as degree of toxicity of its strains. N. I. Mirakhmedov and M. N. Dobrotvorskaya report that there is greater RNA saturation in hypha cells of the fungus Verticillium dahliae, which is highly pathogenic for the cotton plant, and

low RNA content in saprophytic strains. Unfortunately, we found no information on this score referable to A. flavus fungi. However, according to findings made for other species of fungi, the existence of such a link appears to be possible in principle.

Thus, A. flavus is one of the main producers of aflatoxins. It is widely distributed in the world, attacks the most varied objects and, first of all, foodstuffs. For this reason, contamination of foods with aflatoxins is a rather common occurrence in different regions of the world. A. flavus is also widely distributed in the USSR, including Kazakhstan.

Aflatoxins are extremely potent carcinogens, with predominantly hepatotropic orientation, that attack a rather wide range of animals on different steps of the evolutionary ladder, including primates, and they are quite nazardous to human health, both in the toxic and oncogenic respects.

These circumstances make it imperative to pursue broad and in-depth investigations of different aspects of the problem, dealing with distribution, morphology and physiology of one of the main producers of aflatoxins--A. flavus. The data in the literature concerning this question are incomplete and, not infrequently, poorly adapted to the needs of medicine, particular sanitary mycology. Still unanswered is the question of incidence of the aflatoxinogenicity trait among strains of this species. There are no convincing data about the existence of morphological, ontogenetic and cytochemical parallels of aflatoxinogenicity, which could be used as criteria of presence or absence of this trait. Nor are there any sufficiently validated data concerning the feasibility of assessing contamination of a concrete alimentary substrate with aflatoxins on the basis of determining the aflatoxinogenic function of strains isolated from the substrate. Yet such data would alleviate the task of sanitary mycological inspection of foodstuffs, as well as screening of A. flavus strains with the sought trait for research and practical purposes.

It is apparent from the foregoing that purposeful studies on the issues raised are necessary.

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TECHNICAL EQUIPMENT FOR PROFESSIONAL PSYCHOPHYSIOLOGICAL SCREENING OF MILITARY SPECIALISTS

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[Text] The use of methods involving forms for professional screening of military specialists is not always acceptable or expedient (particularly in the case of mass screening) due to the complexity and time required to process the results and delays in decision making. Introduction of instrumental (equipment) methods reduces the screening time. Development of automated systems for group examination and active use of computer technology is a promising direction of development of methods for professional screening.

In recent years, instrument methods of psychophysiological examination for purposes of professional screening began to be used more extensively (K. K. Platonov, 1970; K. B. Zimin, 1971; V. A. Mozin, N. A. Kossova, 1974; Ye. A. Umryukhin, 1976; O. O. Sukhanov, 1977, and others). At the same time, the examination process is not usually entirely automated, although the current status of radio electronics, computer technology and mathematics could solve this problem entirely.

We use the term, automated professional screening, to refer to a psychophysiological examination of an individual where all stages of screening (formation of stimuli, presentation thereof to a subject, recording responses, mathematical processing of results and putting them out in a form convenient for analysis, preparation of recommendations for making a decision as to placing a subject in one of the classification categories) are automated.

At the present time, there are instruments and entire complex systems based on microprocessors and microcomputers. The advantages of such technology are obvious. At the same time, questions arise that require independent investigation. For example, the criteria worked out by A. A. Genkin and V. A. Bodrov (1967) for scoring professional fitness were obtained primarily for form methods [methods requiring forms to be filled out] and in our instance they are unsuitable and require further specification.

Some experimental models of automated systems for professional screening and checking psychophysiological functions (V. N. Zvegintsev, 1971; S. A. Manuylov,

1974), which are based on either stationary computers or logic-information units, have several flaws: large size of equipment and high cost, poor mobility of the system as a whole and difficulty of operation.

There are certain advantages to automated equipment for professional screening that is based on keyboard program computers (PFK-1): they can be used in the field and under laboratory conditions.

When conducting tests with such equipment under real conditions, one can obtain both statistical data to work out screening criteria and check the suitability of the equipment itself for mass screening. Nevertheless, experience has shown (V. A. Mozin, I. F. D'yakonov, 1980) that the weakest point in using them is the limitation on simultaneous group examinations. When subjects are examined successively through psychophysiological tests, even when all stages are completely automated, the process itself is prolonged and limited to the area of use of this equipment.

Refinement of equipment for automated professional screening is proceeding in several directions.

The methodological direction refers to development of new (for new specialties) and definition of existing models and complexes [sets] of professionally important traits (psychophysiological and physiological) that provide for efficient and reliable operator performance. It also includes development of methods and criteria for assessing professional suitability based on the use of parameters of the state of several systems of the body.

The ergonomic direction determines development of equipment for professional screening with consideration of ergonomic requirements, for example, of the geometric dimensions of a console (consideration of athropometric data) and lay-out of display equipment (DE) and controls (C), choice of their shape, dimensions, color, etc.

The principle of standardness, i.e., typing choice and location of DE and C, by means of which stimulus signals affect sense organs and the subject's responses to a given signal occur, is the main principle in developing technical equipment and, first of all, the subject's consoles. Failure to adhere to this principle would lead to obtaining results that cannot be compared from using the same psychophysiological methods based on different computer equipment.

The technical direction refers to development of peripheral equipment (for input and output) for exchange of information between the computer part of the system and the subject's console. Consideration is given to modular assembly of psychophysiological and physiological channels in the system, which make it possible for it to be further developed, etc.

The metrological direction includes the choice and validation of nomenclature, range and accuracy of recorded primary parameters of mental and psychophysiological functions. It provides for development of requirements referable to means of metrological certification and methods of checking the equipment used.

Metrologically supported, unified and standardized parameters for professional screening make it possible to create a universal automated complex with an optimum set of test methods and parameters, in which additional subject consoles can be added, depending on the specific purpose (screening of operators in a specific special field), which make it possible to run the required methods according to programs.

The program-algorithm direction refers to development of software systems for running psychological and psychophysiological tests and classification of the tested group of individuals. The algorithms and programs must meet the main psychological requirements, which are used in ordinary instrument examinations. These requirements are based on the principle of changing the probabilistic structure of stimuli, which provides for varying the probabilities of presenting them in the range of 0 to 1, as well as the principle of random appearance of a stimulus. Only coordinated [complex] work on solving these problems will enable us to have a reliable, objective and operative system for automated professional screening.

The use of general and special principles of constructing automated systems for professional screening and monitoring the condition of an individual enabled us to work out stationary and portable automated systems for psychophysiological screening. They enable us to investigate the functional state of the central nervous system by assessing mental work capacity, on the basis of determination of the time and flawlessness of performance of both the individual elements in the structure of operator work and the aggregate thereof. One can evaluate the state of the motor analyzer on the basis of evaluation of the pace, rhythm and stability of motor action by means of the above systems.

Automated systems consist of the following main components: electronic computer with digital printer of the Consul type, unit that connects the computer with terminals; terminals for delivery of psychophysiological stimuli (audio, photic, symbolic, etc.). The automated system makes it possible to obtain information about an individual's state immediately and objectively, since the results of the tests are put out right after the experiment ends.

Checking [or monitoring] and forecasting professional suitability or psychophysiological state of an operator are effected as follows: according to current values of time parameters of performing operations within T_{fixed}; according to values of X per epoch of analysis with T_{fixed}, with T_{max} and T_{min}; according to quantity of erroneous actions, both overall and distributed according to stimuli and analytic epochs.

As terminals for delivery of stimuli we use, for example, experimental consoles for testing mental work capacity, simple and complex sensorimotor reactions, reflex to time, as well as consoles to examine mechanisms of spatial orientation, reactions to moving object, memory for numbers, etc. Storage of information about the initial and subsequent state of the oeprator in the computer's memory enables us to determine the reliability of differences.

The set of methods used enables us to utilize the required number of parameters obtained as a result of the screening and, knowing the weight coefficients

(which are stored in the computer's memory), the computer calculates the criterion of professional fitness using the following formula:

$$K = a_0 + \sum_{i=1}^{N} a_i x_i,$$

where x_i is the value of parameters; a_0, a_i are weight coefficients; N is the number of screening parameters.

The calculated criterion K is then compared to thresholds K_H and K_B (which are also stored in the computer's memory), and the result of this comparison (conclusion) is printed out. For example:

$$S = \begin{cases} -1 & K < K_H \quad \text{Unfit} \\ 0 & K_H < K \leqslant K_B \quad \text{Prognosis uncertain} \\ +1 & K_B < K \quad \text{Fit} \end{cases}$$

Analysis of differential diagnostic tables given in the instructions for psychological screening shows that one can use the psychophysiological methods run in the automated system to screen operators in different special fields.

On the whole, the automated systems can perform the following main tasks:

1. Detect stable psychophysiological and psychological human traits that are needed for efficient and reliable performance of specific operator work. The combination of these traits constitutes the basis for the requirements of professional psychophysiological screening that are put to applicants for operator jobs.

2. Forecast the possibility of performing a proposed task without accident, with the required efficiency (pre-lift-off [or take-off], pre-cruise inspection). Forecast states that could lead to lower efficiency and reliability of performance.

3. Determine operator endurance of environmental factors differing in magnitude, duration and gradient while conducting ergonomic tests of a military system.

4. Implement medical expertise--determine cause and effect relationship between the functional state of an operator and an accident, malfunction of military materiel or armament complex and other similar instances.

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